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STEM CELL THERAPY FOR MYOCARDIAL REGENERATION

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EXECUTIVE SUMMARY:

Myocardial regeneration through stem cell therapy is a relatively new concept that has the potential to help millions of patients with ischemic heart disease and congestive heart failure. Proof of concept for stem cell-mediated angiogenesis and myocardial regeneration was established in early animal studies; data from subsequent phase I and II human clinical trials are encouraging. Autologous skeletal myoblasts are the most extensively studied stem cells and appear to be relatively safe and efficacious when injected directly into the myocardium, either through an open-chest or percutaneously. The occurrence of arrhythmogenicity remains an issue, however. Other bone marrow stem cell preparations have also shown promise in improving left ventricular function at follow-up, however these improvements may not be sustained. Refinements in the types of cells delivered and the mode of delivery may lead to more substantial and long-lasting results. The use of granulocyte colony-stimulating factor to augment the body's natural mobilization of bone marrow-derived stem cells to damaged myocardium has not been proven to be useful based on clinical studies available to date. Future challenges include determining the appropriate type, dose, timing, and delivery of cells, as well as identifying the subset of patients who are most likely to derive benefit.

Introduction

Despite major advances in the treatment of ischemic heart disease and congestive heart failure (CHF) over the past two decades, morbidity and mortality still remain high. The development of novel treatment strategies is required to further improve clinical outcomes. One such novel approach is the application of stem cell therapy for the treatment of acute myocardial infarction, ischemic congestive heart failure, and refractory angina.

Stem cells are undifferentiated cells, able to renew themselves or replace other cells in a variety of tissues, and ultimately give rise to differentiated, mature cell types. Progenitor cells usually belong to a transitory amplifying population of cells derived from a stem cell and do not have the capacity for sustained undifferentiated self-renewal (1). As work with human embryonic stem cells is limited due to ethical and immunological complications, this discussion will focus on autologous skeletal myoblasts (ASMs) and human adult bone marrow stem cells (unfractionated mononuclear, mesenchymal, and hematopoietic stem cells), as well as bone marrow mobilized stem cells.

Autologous Skeletal Myoblasts

Skeletal myoblasts were the first type of stem cells evaluated in cell-based cardiac repair, and have been extensively studied in the past 14 years. ASMs are mononucleated satellite cells, which are committed progenitor cells that differentiate into mature skeletal myocytes. They do not transdifferentiate into cardiomyocytes. The cells are collected from a skeletal muscle biopsy, purified and propagated in vitro (a process which take about 2- 4 weeks), and then injected into the infracted area of the myocardium.

Following multiple animal studies investigating skeletal myoblast grafting for cardiac repair, the first human case was reported in 2001 by Menasche et al. (2). A patient with ischemic heart failure received a direct epimyocardial injection of ASMs at the time of coronary artery bypass grafting (CABG) into the infarction zone. A subsequent phase I feasibility and safety trial evaluated the direct injection of ASMs into infracted myocardium during CABG in ten patients with severe ischemic cardiomyopathy (3). The procedure was determined to be feasible, but safety was unclear due to potential arrhythmogenicity. While the study was not powered or designed to test efficacy, there was an improvement in mean New York Heart Association (NYHA) functional class (from 2.7 to 1.6; $p < 0.0001$) and left ventricular ejection fraction (LVEF) at a mean follow-up of 11 months (from 24% to 32%; $p < 0.02$). More recently, 30 patients with ischemic cardiomyopathy underwent ASM epimyocardial injection concurrent with CABG or left ventricular assist device (LVAD) implantation (4). The procedure was successful and well tolerated, with an increase in LVEF and tissue viability (determined by positron emission tomography (PET) scanning and gadolinium magnetic resonance (MR) imaging delayed hyperenhancement) in those having CABG, as well as histological confirmation of skeletal myoblast survival and engraftment within the infracted myocardium in those successfully bridged from LVAD to heart transplantation.

An alternative method of ASM delivery, percutaneous transplantation by transendomyocardial injection, was presented in a phase I safety and feasibility study with 6 month follow-up (5). Five patients with a prior anterior wall myocardial infarction, depressed LV function, and NYHA functional class ≥ 2 were enrolled. All procedures were uneventful, but arrhythmogenicity during follow-up was again noted (6). LVEF was improved from baseline at 3 months (from 36 % to 41 %; p = 0.009), but at the 6 months follow-up there was no difference (p = 0.23). At 3 months, there was also significantly increased wall thickening at the target areas and less wall thickening in remote areas by MRI regional wall analysis.

A randomized trial of this catheter-based delivery method is currently underway at the Arizona Heart Institute. Twenty-four patients with a history of myocardial infarction and symptomatic congestive heart failure are being randomized to receive either intramyocardial injection using the NOGA® XP Cardiac Navigation System (Biosense-Webster, Inc., Israel) (Figure 1) and the NOGA guided Myostar™ injection catheter (Cordis, Miami Lakes, FL) (Figure 2) or medical therapy alone. The NOGA system creates a three-dimensional real-time dynamic reconstruction of the endocardium of the left ventricle and through electromechanical mapping allows identification of targeted tissue, such as an infarction zone (Figure 3). The trial is ongoing and, thus far, the procedure appears to be safe and feasible.

Bone Marrow Stem Cells

Several bone marrow clinical trials have been performed in both acute myocardial infarction (MI) and ischemic congestive heart failure (Table 1).

Mononuclear bone marrow (MNBM) stem cells.

MNBM stem cells are unfractionated autologous bone marrow cells which are generally delivered via an intracoronary route. Early clinical investigations suggested that insertion of autologous bone marrow cells into the infarcted coronary artery was feasible and safe after acute MI. TOPCARE-AMI (Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction), a non-randomized pilot trial, transplanted MNBM cells into the infarcted artery of 20 patients with reperfused acute MI (7). The investigators found that cell transplantation was associated with a significant increase in LVEF and a significant decrease in end-systolic left ventricular volume at both 4- and 12-month follow-up. No evidence of an acute inflammatory response, ischemic damage, or malignant ventricular arrhythmia from the cell transplantation was present. The first randomized trial, BOOST (Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration), included 60 patients with recent ST-elevation MI randomized to either optimal medical treatment and PCI together with or without intracoronary injections of autologous MNBM cells (8). The procedure was not associated with adverse clinical events. At 6 months, the cell therapy group had a significant increase in LVEF by MRI compared with the control group (50% to 57% vs. 51% to 52%; p = 0.0026). At 18 months, however, there was no longer a difference (p = 0.27) (9). It was concluded that a single-dose of intracoronary MNBM cells sped up the recovery of LVEF following MI, but ultimately did not provide long-term benefit. The lack of long-term benefit may be due to the relatively low number of CD34+ cells in the cell preparation, as these cells display higher retention in the infarcted myocardium compared with unselected bone marrow cells (10). An increased dose of CD34+ cells was evaluated in the REPAIR-AMI (Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction) trial, a large randomized, double-blind, multi-center study involving 204 patients with ST-elevation MI randomized to autologous intracoronary MNBM cell infusion or placebo through a sham procedure at 4 days post-MI (11). At 4 months there was more improvement in LVEF from baseline in the treatment group compared with the placebo group (5.5 % vs. 3.0 %; $p = 0.014$). This improvement was more marked in those with a lower baseline EF (< 49%) and those treated later (> 5 days) after their infarct.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are a rare population of cells found in mature bone marrow (0.05 % of the total cell population); however, like skeletal myoblasts, one donor sample can be expanded in vitro to treat hundreds of patients. MSCs are progenitor cells able to differentiate into different kind of cells such as osteocytes, adipocytes, and even occasional cardiomyocytes after intracoronary infusion. In addition, MSCs lack histocompatibility antigen on their surface which makes them theoretically less immunogenic and attractive for allogenic use (12). A randomized, double-blind, multi-center, placebo-controlled phase 1 safety and efficacy trial was recently completed using a universal adult stem cell drug candidate (Provacel™, Osiris Therapeutics, Inc., Baltimore, MD). This drug uses CD45-, CD105+, and CD166+ MSC, is allogenic (not patient specific), and is given through intravenous infusion. Fifty-three patients successfully treated with percutaneous revascularization following an acute MI were randomized to the drug versus placebo and early safety data looks favorable.

Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are 10 to 20 times more abundant than the mesenchymal stem cells, comprising 1-2 % of the bone marrow. There is evidence that when transplanted into the (murine) myocardium, HSCs may transdifferentiate into cardiomyocytes and blood vessels (13). In particular, hematopoietic CD34+ stem cells are thought to be more likely to form angiogenic precursors than undifferentiated bone marrow cells, thus potentially being able to regenerate damaged microcirculation. Following promising phase I results, Baxter Healthcare Corporation (Deerfield, IL) has initiated the first human phase II stem cell therapy trial in the U.S. using hematopoietic CD34+ stem cells. In this double-blind, placebo-controlled, multi-center study approximately 150 adult patients with untreatable refractory angina will be randomized to intramyocardial injection of autologous CD34+ cells versus placebo using the NOGA system. To create a concentrated preparation of CD34+ cells, the company is using a specialized sorting system that selects out CD34+ cells from granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood, rather than obtaining the cells directly from the bone marrow.

Bone Marrow Stem Cell Mobilization with Granulocyte Colony-Stimulating Factor (G-CSF)

Following an MI, bone marrow-derived stem cells spontaneously mobilize to the heart where they differentiate into cardiac cells; this process may be enhanced with G-CSF (Table 2). In the FIRSTLINE-AMI (Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction) trial, 50 patients who had been successfully stented following an acute ST-elevation MI were randomized to receive standard of care with or without subcutaneous G-CSF at 10 μg/kg for 6 days (14). There was evidence of G-CSF-induced cell mobilization and the drug was well tolerated. At 4 months, resting wall motion score index, left ventricular end-diastolic diameter, and ejection fraction were all significantly improved in the treated group.

The largest and most recent trial, the REVIVAL-2 (Regenerate Vital Myocardium by Vigorous Activation of Bone Marrow Stem Cells) trial, was a randomized, double-blind, placebo-controlled study of 114 patients who presented with an ST-elevation MI and were successfully reperfused with percutaneous coronary intervention (15). These patients were randomized to subcutaneous injections of either 10 μg/kg of G-CSF or placebo for 5 days. There was evidence of significant mobilization of stem cells in the treated group, and no major differences in adverse events between either of the groups. However, unlike previous studies, this trial found no effect of G-CSF on mean infarct size (as measured by technetium Tc 99m sestamibi scintigraphy, $p = 0.56$) or LVEF (as measured by mean residence time, $p = 0.14$). In addition, there was no increased rate of in-stent restenosis as had been previously reported (16). Given the results of this trial, it is unlikely that any further large clinical trials of this nature will be performed.

Stem Cell Delivery

The best way to deliver stem cells remains unknown. Various routes, including intravenous (IV), intracoronary (IC), via the coronary sinus, and intramyocardial (IM), have been investigated in combination with different endomyocardial injection techniques for either direct injection during open-chest surgery or percutaneously delivery. To compare the efficacy of different delivery modalities, human radiolabeled peripheral blood mononuclear cells were injected via IC, IM (open-chest), and interstitial retrograde coronary venous delivery 6 days after a myocardial infarction was induced in swine (17). The IC route was relatively easy to use and did not require any significant training, but the retention of cells within the myocardium was only 2.6 % compared with 11% when the IM method was used ($p < 0.05$). Retention following injection via the coronary sinus was also low (3.2%). While the IM injection had the best retention of the three modalities, it also displayed the greatest amount of variability in delivery efficiency.

Conclusions

Cell-based cardiac repair is a very promising new frontier in the treatment of acute myocardial infarction, ischemic congestive heart failure, and refractory angina. The idea of using autologous or allogenic stem cells to repair injured myocardium is gathering momentum with data mounting from basic science studies, animal research, and phase I and II clinical trials. Stem cell therapy appears to be relatively safe and there are trends toward benefit, but multiple unanswered questions remain in terms of type, dosing, timing, and delivery of stem cells. In addition, new concepts such as stem cells causing a paracrine effect, i.e. producing beneficial growth factors, cytokines, and other local signaling molecules, continue to emerge. Further basic science work along with eventual phase III clinical trials will shed more light about efficacy and hopefully move this concept of regenerative cardiology from bench to bedside.

Table 1. Bone Marrow Clinical Trials

Study	N	# of cells (x 10^6)	Mean infarct duration (hours)	Peak CK	Days to cell delivery	Safety	LVEF (BL)		LVEF (FU)	
							Con	Tx	Con	Tx
Strauer et al. Circulation 2002;106:1913	40	46	11	1156	9	yes	54	55	56	66
Assmus et al. (TOPCARE-MI) Circulation 2002;106:3009	28	238/13	23	1381	4.5	yes		44		49
Wollert et al. (BOOST) Lancet 2004;364:141	60	2460	9.8	2968	5.7	yes	51.3	50	52	56.7
Janssens et al. Lancet 2006;367:87	67	304	3.7	2255	1	yes	46.9	48.5	49	51.8
Lunde et al. (ASTAMI) Scand Card J 2005;39:150	50		4.4		5	yes	46	46	48	47
Aviles et al. Rev Esp Card 2004;57:191	33	78			13	yes		51		57
Schachinger et al. (REPAIR-AMI) unpublished	204		4.5		4	yes	47	48	50	54
Bartunek et al. Circulation 2005;112:I178	35	12.6			11.6	Restenosis	44.3	45	48.6	52.1
TOTAL	517	28-2460	3.7-23	1156-2968	1-9	Yes	48.1	48.4	50.6	54.2

N = number of patients randomized, CK = creatinine kinase, Con = control, Tx = treatment, LVEF (BL) = left ventricular function at baseline, LVEF (FU) = left ventricular function at follow-up.

Table 2. G-CSF Clinical Trials in Acute MI

Study	Cell Type	Enrolled	Treatment	Control
Kang et al. (MAGIC) Lancet 2004;363:751	IC peripheral blood stem cells + G-CSF	27	10 cell only 10 G-CSF + cell	7
Zohlnhofer et al. (REVIVAL-2) JAMA 2006;295:1003	10µg/kg G-CSF for 5 days	114	56	58
Ince et al. (FIRSTLINE-AMI) Circulation 2005;112:3097	10µg/kg G-CSF for 6 days	50	25	25
Ripa et al. (STEMMI) Circulation 2006;113:1983	10µg/kg G-CSF for 6 days	78	39	39
Valgimigli et al. Eur Heart J 2005:26:1838	5µg/kg G-CSF for 4 days	20	10	10
	Total	**269**	**130**	**129**

Figure 1. NOGA® XP Cardiac Navigation System

Figure 2. NOGA® guided Myostar™ Injection Catheter

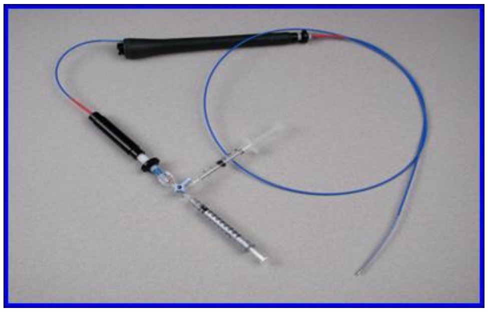
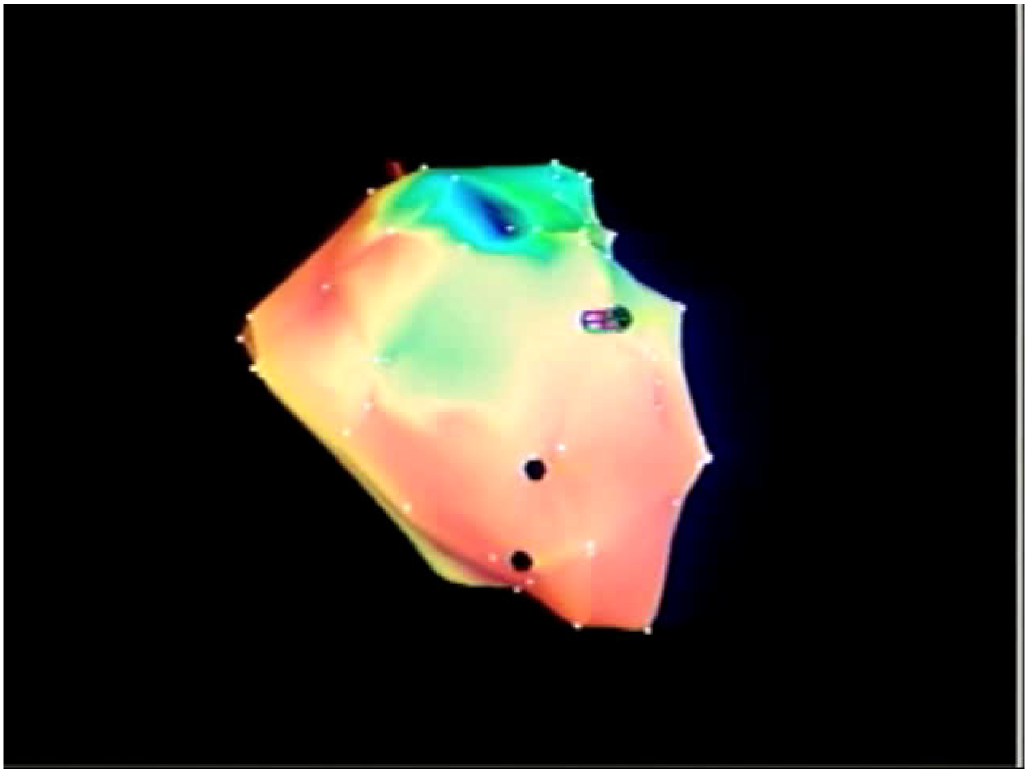


Figure 3. NOGA® Mapping of the Left Ventricle: Red indicates the infarction zone; the two black dots point to the stem cell transplantation sites.



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