Stem cell therapy for refractory angina

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Abstract

Stem cells (SC) have demonstrated significant potential for regeneration of ischemic myocardium in both animals and humans, primarily after acute myocardial infarction. Recently, SC therapy for unstable angina pectoris, to include intractable chest pain and non-revascularizable coronary disease, has displayed clinical benefit within this subset of patients that are often refractory to medical therapy and poor candidates for reperfusion strategies, and thereby urgent need for novel therapies. Few human clinical trials have been completed to date with positive yet variable results due to inconsistencies in trial design rendering them difficult to interpret for clinical practice. Herein, a summary of all clinical trials of SC therapy in refractory angina is provided with pertinent findings from each study.

Introduction

There is increasing evidence that stem cells (SC) can be an effective therapy for regeneration of ischemic myocardium. Numerous clinical trials have shown an improvement in cardiac function with the administration of SC after acute myocardial infarction.[1] However, SC therapy in patients with unstable angina pectoris (Figure 1), to include intractable chest pain and non-revascularizable coronary disease, has only been sparsely studied. Since these patients are often refractory to medical therapy and poor candidates for reperfusion strategies, there is immense need for novel therapies.

Preclinical animal studies have demonstrated that injection of SC, obtained from autologous bone marrow (BM), into ischemic areas in chronic myocardial infarction (MI) models results in improved cardiac function and increased vascularity.[1] The proposed mechanisms behind these findings are angiogenesis and improved collateral circulation in the ischemic area. There is evidence to support that BM releases factors such as vascular endothelial growth factor (VEGF) and chorioallantoic membrane (CAM) that promote vasculogenesis.[2,3] Furthermore, there is data to suggest that injected BM-SC can lead to regeneration of infarcted myocardium by differentiating into a variety of cells including myocytes, endothelium, and smooth muscle cells.[4,5] These findings have led to a number of clinical trials geared towards establishing the safety and efficacy of SC therapy as a last resort in patients with refractory angina pectoris.

Majority of SC studies for refractory chest pain have primarily used autologous adult BM cells, although other sources such as peripheral blood and umbilical cord blood have also been evaluated. However, due to the low risk of rejection as compared to umbilical cord blood SC and the high yield of cells as compared to peripheral blood SC, adult BM-SC have prevailed as the most popular source. Human trials for chronic MI have also evaluated various methods of SC delivery, with the most common being direct intramyocardial (IM) injection with and without indirect mobilization of stem cells using granulocyte-colony stimulating factor (G-CSF). Alternate methods of delivery include intracoronary (IC) infusion, transcoronary sinus (TCS) infusion, and transmyocardial laser revascularization (TMLR) with direct IM injection.

Direct intramyocardial injection

The safety and feasibility of IM injection of SC (Figure 2) in patients with refractory angina via cardiac catheterization has been established in multiple non-randomized trials[6-8] as summarized in Table 1. Tse *et al.*[6] first reported it in eight patients with no adverse effects. These patients were followed for only three months and demonstrated a reduction in anginal symptoms despite no significant improvement in left ventricular ejection fraction (LVEF). In addition, the study revealed a decrease in percentage of ischemic myocardium as compared to baseline after three months. Subsequently, Brigouri *et al.*[7] studied nine patients who were not eligible for revascularization and showed improvement in anginal symptoms within one year of follow up. Moreover, half of these patients demonstrated decreased severity and extension of ischemic area. Fuchs *et al.*[8] administered BM-SC via IM injection to 27 patients with unrevascularizable coronary disease, showing an improvement in anginal symptoms and improved stress-induced perfusion scores at one year. Additionally, this study compared characteristics of BM-SC administered to responders and non-responders and found that there was no statistically significant difference in cellular composition including percentage of $CD34^+$

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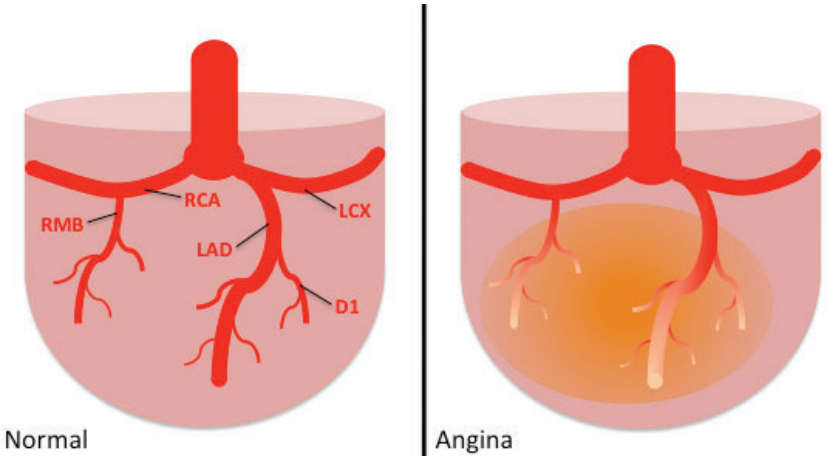

Figure 1. Overview of unstable angina. Coronary arterial circulation of the ventricles in a normal heart (left), with the major arteries highlighted: left circumflex (LCX), left anterior descending (LAD), first diagonal branch (D1) of the LAD, right coronary artery (RCA) and the right marginal branch (RMB) of the RCA. Schematic representing chronic anterior wall ischemia (right) due to unrevascularizable coronary artery disease.

Table 1. Summary of clinical trials using stem cell therapy for refractory angina.

Study	Trial design	Groups (n)	Cell type	Cell count	Follow up (months)	Primary end point	Change in EF primary end point
Tse *et al.*[6]	NRNC	BM Intramyocardial injection (8)	BM (mononuclear cells)	Unspecified (3.2% $CD34^+$)	3	MRI wall thickening	Improvement in wall thickening by 11.6%
Briguori *et al.*[7]	NRNC	BM Intramyocardial injection (10)	BM (mononuclear cells)	32.6×10^6 (4.0×10^6 $CD34^+/CD45^+$)	12	Ischemic area by SPECT and PET	Decreased severity and extention of ischemic area in 4 of 8 patients
Fuchs *et al.*[8]	NRNC	BM Intramyocardial injection (27)	BM (mononuclear cells)	28×10^6 (2.4% CD34-containing)	12	Stress myocardial perfusion score by SPECT (3 mos) CCS angina score (1 year)	Improved stress induced perfusion score from 2.2 to 1.7 Improvement of CCS from 3.2 to 2.2
Beeres *et al.*[9]	NRNC	BM Intramyocardial injection (25)	BM (mononuclear cells)	84×10^6	12	Stress myocardial perfusion score by SPECT	Ischemic segments decreased from 4.7 to 1.6
Tse *et al.*[11]	RC	BM Intramyocardial injection (10, 9) PL Intramyocardial injection (9)	BM (mononuclear cells)	2×10^6 ($0.79\times10^6$$CD34^+$) 1×10^6 ($1.38\times10^6$$CD34^+$)	6	Exercise treadmill time	Increase in exercise time from 393 s to 464 in BM treated group Decrease in exercise time from 439 to 383 in control
Ramshorst *et al.*[12]	RC	BM Intramyocardial injection (24) PL Intramyocardial injection (25)	BM (mononuclear cells)	100×10^6	6	Stress myocardial perfusion score by SPECT	Improved summed score from 23.5 to 20.1 in BM group Improved summed score from 24.8 to 23.7 in PL group
Losardo *et al.*[17]	RC	BM Intramyocardial injection + GM-CSF (6,6,6) PL Intramyocardial injection + GM-CSF (9)	BM ($CD34^+$ cells only)	5×10^4 1×10^5 5×10^5	6	Angina Frequency/ Nitroglycerin Use	Improvement of angina in groups that received combination therapy
Vicario *et al.*[20]	NRNC	BM Transcoronary Sinus Infusion (15)	BM (mononuclear cells)	0.09×10^8/kg	12	CCS angina score, stress myocardial perfusion by SPECT, Coronary Angiography	Improvement of CCS from 3.0 to 1.6, Improvement in stress summed score from 19.9 to 13.6, More collaterals on angiography
Reyes *et al*[22]	NRNC	Transmyocardial Laser Therapy + BM Intramyocardial injection (14)	BM (mononuclear cells)	81.3×10^6 (0.6×10^6 $CD34^+$) (0.37×10^6 $CD133^+$)	Mean of 7 months	CCS angina score	Improvement of CCS from 3.2 to 1.4

RC, randomized controlled; NRNC, non-randomized non-controlled; BM, bone marrow; PL, placebo.

cells and rates of growth factor secretion.[8] Beeres *et al*.[9,10] further described improvement in quality of life (QOL) scores and stress-induced perfusion scores in patients receiving BM-SC via IM injection after one year. All of these studies were carried out without any adverse outcomes attributable to the intervention.

The first randomized controlled trial involving IM injection of BM-SC for refractory chest pain, PROTECT-CAD,[11] enrolled 19 patients in the experimental group, which had low-dose (9 patients) and high-dose (10 patients) subgroups depending on the number of BM-SC delivered, and an additional 9 patients in the control group, that received placebo injections, followed for a total of 6 months. Targeted SC injection was performed using the Myostar catheter (Biosense-Webster Inc., Diamond Bar, CA, USA) after left ventricular electromechanical mapping using the NOGA catheter (Biosense-Webster Inc.). Compared with the control group, patients that received SC therapy showed a statistically significant progression in exercise treadmill time, increase in ejection fraction (+5.4%) after 6 months, and improvement in New York Heart Association (NYHA) functional class. Canadian Cardiovascular Society (CCS) survey score decreased similarly in both experimental and control groups. There was no statistically significant difference in total exercise time between the low and high-dose groups and improvement was not found to be correlated with quantity of BM-SC delivered.

The largest randomized controlled trial to date published by Ramshorst *et al*.,[12] was carried out using 50 patients with the primary outcome being 17-segment score in a stress myocardial perfusion study. Electromechanical mapping and IM SC delivery was again performed using the NOGA and Myostar catheters. This study followed patients for only 6 months with stress myocardial perfusion studies and left ventricular function being assessed at only 3 months. There was significant improvement in the summed stressed perfusion score at 6 months and ejection fraction at 3 months in the experimental group while no change was seen in the control group. Moreover, CCS and QOL scores were also significantly higher at 6 months in the experimental group as compared to controls.

The treatment of patients with refractory angina using IM injection of SC has demonstrated improvement of anginal symptoms and increased perfusion of chronically ischemic myocardium in multiple trials carried out to date,[6-12] of which only two are randomized-controlled trials[11,12] with the largest trial enrolling only 50 patients.[12] The variability in findings among these studies are likely due to the disparity in study design (differing end points, follow up times, quantity of cells delivered, and composition of cells delivered). More long-term trials are needed as the longest follow up is for 12 months. The upcoming FOCUS (First Mononuclear Cells injected in the US) trial[13] will examine transendocardial delivery of SC in a randomized, controlled trial where isolated BM-SC will be delivered using the NOGA and Myostar catheters and followed for 5 years. The clinical endpoints include change in maximum oxygen consumption, left ventricular end systolic volume as measured by echo, and change in ischemic defect size as assessed by single-photon emission computed tomography (SPECT). This trial will present long-term data regarding the efficacy of IM injection of BM-SC.

Combination of indirect mobilization and direct intramyocardial injection

Animal models have shown that cytokines can be used to induce mobilization of BM-SC (Figure 3) in infarcted myocardium[14] resulting in increased angiogenesis and coronary collateralization.[15] The end result in animal MI models has been improved left ventricular function and survival.[13] While many clinical trials exist examining the use of cytokine therapy, most commonly G-CSF in acute MI,[1] no clinical trials have yet been performed using cytokine therapy alone for refractory angina. Animal models have also displayed an improvement in cardiac function in chronic MI models treated with VEGF secondary to improved angiogenesis in ischemic myocardium.[16] However, no clinical trials evaluating the use of VEGF in unstable angina pectoris in humans have been completed.

There has been a single randomized controlled trial by Losordo *et al*.,[17] examining combination therapy of G-CSF and IM injection of SC, with one group receiving IM CD34$^+$ BM-SC and subcutaneous G-CSF, but the other group receiving only subcutaneous G-CSF. Improvements were observed in frequency of angina

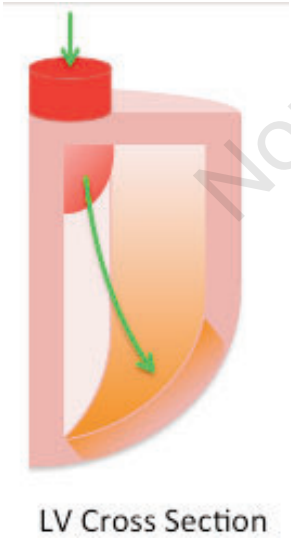

Figure 2. Direct intramyocardial (transendocardial) injection. Image showing the inside of the left ventricle. An injection catheter is passed into left ventricle using flouroscopy and stem cells are injected transendocardially into the ischemic myocardium (shaded).

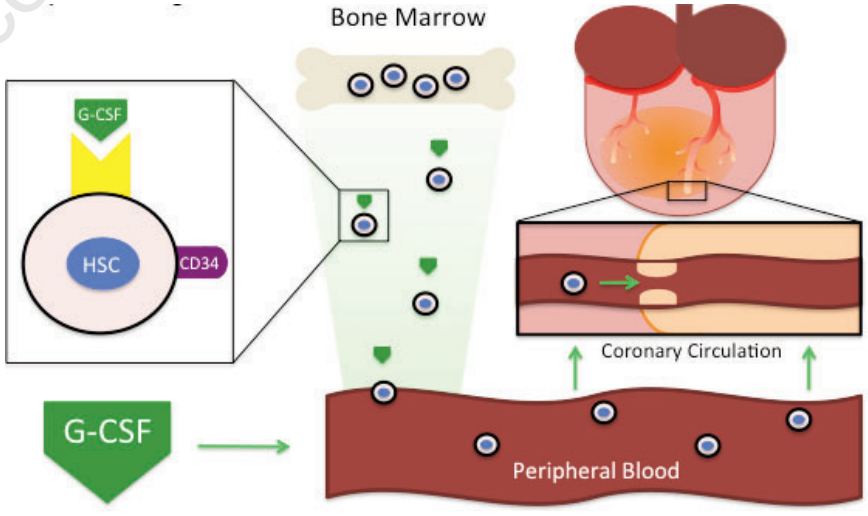

Figure 3. Administration of exogenous granulocyte-colony stimulating factor (G-CSF) mobilizes quiescent hematopoietic stem cells (HSCs) from the bone marrow to peripheral circulation. Mobilized HSCs home to damaged tissues, where they mediate neovascularization and improve symptoms of unstable angina. Magnified on the left is an HSC, showing the G-CSF receptor/ligand interaction, and CD34, a surface marker signifying pluripotency in the vascular lineage.

symptoms, CCS angina scores, nitroglycerine use, and exercise time at 3 and 6 months in the combination group. SPECT perfusion imaging was also done but the findings were inconsistent: the automated summed perfusion scores improved in both groups, but those receiving combination therapy showed greater improvement; and visually estimated summed perfusion scores were slightly better in the combination group at 3 months but then better in the G-CSF only group at 6 months.

One could conclude from this study that since the control group received only G-CSF and displayed some improvement, that there could be a role for G-CSF administration for patients with refractory unstable angina. This study did not set out to determine if administration of G-CSF could improve ischemia in refractory chest pain as both groups received this therapy. While the improvement seen in the control group cannot be attributed solely to G-CSF administration, the group that received G-CSF without SC therapy did show improvements in angina symptoms, CCS angina scores, and exercise time as compared to their baseline values. One could also hypothesize that G-CSF administration in combination with BM–SC therapy could have a synergistic effect since patients that received the combination showed greater improvement than those that received GM-CSF alone. However, more studies are needed to prove this concept. The authors further divided the patients receiving SC therapy into three groups based on number of $CD34^+$ cells received and found no dose dependent difference between the three groups.

Intracoronary injection

A single clinical trial has been carried out examining IC injection of SC alone (Figure 4) in intractable angina, wherein Wang *et al*.[18] treated 56 patients with IC infusion of BM-SC, while another 56 patients received IC injection of saline in a randomized, controlled fashion. BM-SC were isolated and selected for $CD34^+$ cells, which were then delivered via IC infusion. This trial showed that IC injection of SC in refractory angina was safe as there were no serious adverse events, and also demonstrated efficacy, as there was a reduction in frequency of angina episodes in the experimental group. There was also improvement in nitroglycerine use, exercise time, and CCS class compared to the control group.

Combination of indirect mobilization and intracoronary injection

Only one trial to date has combined indirect mobilization of SC with IC injection. In the GAIN I (G-CSF in Angina patients with IHD to stimulate Neovascularization) trial,[19] patients were pre-treated with G-CSF for 6 days and subjected to exercise stress tests at day 4 and 6 of G-CSF therapy in order to activate myocardial chemokine expression and chemokine-mediated homing of mobilized progenitors to the ischemic myocardium. At the 3rd month follow up, these patients displayed improvement in angina frequency score and physical limitation scores, although 4 out of 20 patients had to be withdrawn from the study due to adverse events. At 3 months, all patients received a second treatment of G-CSF followed by leukapheresis of peripheral blood. Ten of these patients were randomized to receive IC infusion of $CD133^+$ enriched leukapheresis product, while the other 6 patients received leukapheresis product without cell enrichment. Three months after this intervention (6 months after enrollment), the patients continued to show improvement in angina frequency and physical limitation scores. While this study showed efficacy, it was marked with a high number of adverse events with 4 troponin-positive events and 2 episodes of thrombocytopenia. Moreover, there was no improvement seen on technetium Tc-99m sestamibi imaging at 6 months and no additional benefit in the group receiving $CD133^+$ enriched cells.

Transcoronary sinus administration

Only one clinical trial has been completed studying the administration of autologous BM-SC via TCS infusion (Figure 5). This method of SC delivery was initially shown to improve cardiac function in a porcine model.[16] In the human trial published by Vicario *et al*.,[20] 15 patients received autologous BM-SC via TCS delivery in a non-randomized, non-controlled fashion. TCS delivery was performed using percutaneous cannulation and subsequent SC delivery over 5 minutes during balloon occlusion of the coronary sinus for 15 minutes. At one year, patients improved QOL scores and CCS angina scores; 12 of 15 patients displayed an improvement in perfusion imaging; and 10 of 15 patients showed more collateral vessels on angiography. No other clinical trials have examined this route of SC administration.

Transmyocardial laser revascularization and intramyocardial injection

TMLR therapy for patients with ischemic coronary artery disease not amenable to revascularization (Figure 6) has been shown to be superior to maximal medical therapy in randomized trials.[21] The synergistic effects of TMLR and BM-SC therapy was evaluated in 14 patients by Reyes *et al*.[22] in a non-randomized fashion with an average follow up of 7 months. TMLR and SC injections were carried out through a surgical left antero-lateral thoracotomy incision at the fifth intercostal space and a Phoenix combination delivery system (Cardiogenesis Corp., Irvine, CA, USA) used to create laser channels in the ischemic myocardium and simultaneously inject SC into the border zones of these channels. The authors observed a significant improvement in angina class scores, which was the primary outcome measured after the intervention. No imaging modalities were used to assess degree of ischemia in this study.

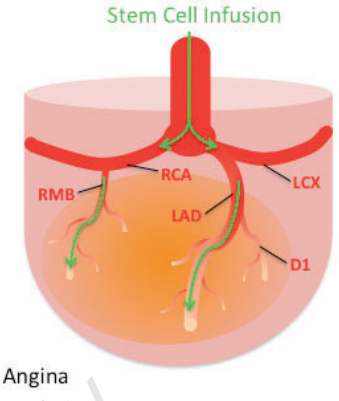

Figure 4. Intracoronary administration of stem cells for unstable angina. The diagram shows only the ventricles and their circulation: the right coronary artery, the left anterior descending coronary artery, and the left circumflex artery. For stem cell delivery to the afflicted vessels, an over-the-wire balloon angioplasty catheter is advanced into the coronary artery. The stem cells are then infused into the coronary circulation and can aid in the revascularization of ischemic myocardium.

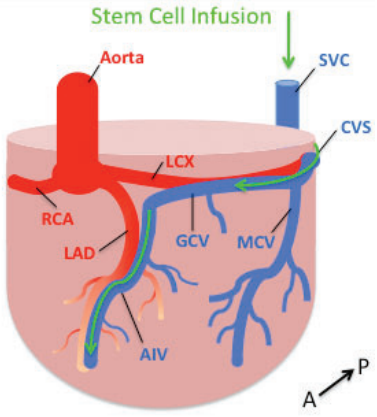

Figure 5. Retrograde perfusion of the coronary venous sinus. The diagram shows only the ventricles and their circulation: the right coronary artery (RCA), the left anterior descending coronary artery, the left circumflexartery, the great and middle cardiac veins, and the anterior interventricular vein (AIV). For stem cell delivery, an over-the-wire balloon angioplasty catheter is advanced into the superior vena cava or into the inferior vena cava (*not shown*). The balloon is inflated and cells are infused under pressure retrograde to coronary venous drainage. Cells flow through the CVS to the GCV on the posterior left ventricle, finally entering the AIV on the anterior wall of the left ventricle. Here, stem cells can reach the affected myocardium and aid in revascularization.

Discussion

Animal models have demonstrated a clinical benefit in the treatment of unstable angina pectoris with BM-SC. Clinical trials have also displayed a benefit in humans; however, very few randomized controlled studies exist as outlined in Table 1. Moreover, there has been an absence of negative published data on SC therapy in refractory angina and no systemic reviews or meta-analyses evaluating the feasibility and value of SC therapy in this subgroup of patients to date although they are abundant within the subgroup of patients with acute MI.[1]

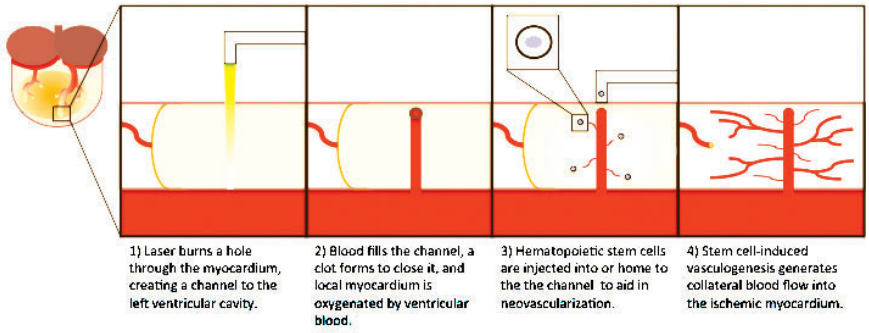

Figure 6. Transmyocardial laser revascularization.

Mechanism of action

While it is evident from multiple small studies that direct injection of BM cells into ischemic myocardium results in a clinical benefit for patients with refractory chest pain, the mechanism of action remains unknown. Multiple theories have been proposed and supported by animal models and the mechanism of action may be multifactorial: direct effects due to acquisition of vascular or cardiac lineage and differentiation into vascular endothelium or functional cardiac myocytes;[23] or indirect effects due to secretion of paracrine cytokines and angiogenic factors leading to proliferation of surrounding myocardial tissue and neovascularization.[24] Direct effects may depend on the specific type of SC as well as on the local environmental niche of the endogenous tissue while indirect effects may be a result of increased expression of chemokines such as G-CSF, SDF-1, or VEGF in the ischemic myocardium. These hypotheses have been difficult to confirm in human trials since tracking cellular differentiation is particularly difficult.[25]

Lineage of stem cells

The stem cell lineage may determine its ability to differentiate into various cell types. Human trials on patients with refractory unstable angina have exclusively used adult BM-SC. However, animal models have demonstrated functional improvement in infarcted myocardium via implantation of SC from a variety of cell lines including embryonic,[26] skeletal myoblasts,[27] cardiac resident progenitors,[28] peripheral blood,[29] and umbilical cord blood.[30-32] While preliminary human studies have been conducted with peripheral and umbilical cord SC in acute MI,[1] no such trials exist for refractory angina.

Characterization of stem cells

SC composition can vary greatly based on cell-surface molecules and mode of isolation. Studies have shown that CD34$^+$ and CD133$^+$ cells contribute to improved neovascularization and cardiac regeneration. Compositions of administered BM stem cells have been inconsistent in human trials for refractory chest pain as outlined in Table 1. Only one trial attempted treating patients with different doses of CD34$^+$ cells; however their relative percentage was the same and no difference was observed in outcomes.[10] The GAIN I trial compared IC infusion of CD133$^+$ enriched cells to leukapheresis product not enriched with a specific cell type, and observed no difference between groups.[19] Future studies with larger sample size are needed to determine if a subgroup of SC with a specific marker have a greater potential for improving cardiac function.

Number of cells

A dose dependent effect with number of BM-SC delivered has not been observed, although few trials have examined this and only with small sample sizes.[11,17] The optimal number of SC needed for therapeutic benefit remains unclear and is reflected in the inconsistencies in cell numbers in study design.

Methods of delivery

The most common mode of SC delivery for patients with refractory angina has primarily been via transendocardial IM injection.[6-12] One study compared G-CSF administration alone with a combination of BM-SC injection and G-CSF administration.[17] Other modes of delivery seen in very limited numbers include IC infusion,[18] combination of indirect mobilization and IC infusion,[1] TCS infusion[20] and TMLR with simultaneous IM injection of BM-SC.[22] Intracoronary infusion of stem cells has not been used as a mode of delivery in clinical trials for patients with unstable angina since these patients are generally not amenable to coronary revascularization and any SC administered would be unlikely to reach ischemic myocardium in contrast to clinical trials for patients with acute MI, where revascularization strategies make reperfusion of ischemic myocardium and delivery of SC feasible.[1]

Timing of delivery

Timing of SC delivery for optimal therapeutic benefit is difficult to assess as no studies have addressed this issue within this population. It may be intuitive that patients with recent onset refractory angina may respond better to SC therapy than those that have had a prolonged course due to less viable myocardium and given that these patients are generally not candidates for reperfusion strategies, timing the therapy with another necessary intervention such as angioplasty is not possible.

Follow-up

The timing of follow-up in clinical trials has been variable as listed in Table 1, with the longest follow up duration being 12 months. Most studies have occurred in the past four years, rendering it difficult to accumulate long-term data. Additional information will be gathered on prolonged effects of BM-SC therapy as more time elapses since the completion of these clinical trials.

Tracking stem cell engraftment and cellular imaging

Human SC trials have been limited by the inability to effectively track the administered cells. The ability to image cells after their delivery would allow investigators to verify the most effective mode of delivery, confirm engraftment, and monitor the fate of these cells with much greater certainty. Current cellular imaging strategies include labeling cells with specific markers *in vitro* prior to transplantation or indirect labeling of cells with imaging reporter genes transduced into the cell before transplantation.[33] Both methods have their limitations as neither provides both qualitative and quantitative data about the transplanted cells. Direct labeling methods use imaging modalities such as positron emission tomography (PET), SPECT, and magnetic resonance imaging (MRI), but are limited by the short half-lives of tracers.[34] In addition, these imaging modalities are not sensitive enough

to pick up individual cells *in vivo* in larger animal models or humans.[35]

Future direction

The use of SC for refractory angina lingers in infancy as very few randomized, controlled trials have been completed to date. While current data suggests that adult BM-SC can improve cardiac function in patients with refractory angina pectoris, primarily in non-revascularizable coronary disease, many questions remain to be answered: optimal technique of delivery; type of SC; method of preparation; dosage of SC; and timing of therapy. Ongoing and future clinical trials including recently completed Phase I/II studies advancing to Phase II[113,17,36,37] comparing SC therapy to placebo are needed before it can become a widespread option of therapy for patients with refractory angina.

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