Human bone marrow-derived adult stem cells for post-myocardial infarction cardiac repair: current status and future directions

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ABSTRACT

Stem cell-based cell therapy has emerged as a potentially therapeutic option for patients with acute myocardial infarction (AMI) and heart failure. With the completion of a number of trials using bone marrow (BM)-derived adult stem cells, critical examination of the overall clinical benefits, limitations and potential side effects of this revolutionary treatment will pave the way for future clinical research. At present, clinical trials have been conducted almost exclusively using BM stem cells. The primary endpoints of these trials are mainly safety and feasibility, with secondary endpoints in the efficacy of post-myocardial infarction (MI) cardiac repair. Intervention with BM-derived cells was mainly carried out by endogenously-mobilised BM cells with granulocyte-colony stimulating factor, and more frequently, by intracoronary infusion or direct intramyocardial injection of autologous BM cells. While these studies have been proven safe and feasible without notable side effects, mixed outcomes in terms of clinical benefits have been reported. The major clinical benefits observed are improved cardiac contractile function and suppressed left ventricular negative remodelling, including reduced infarct size and improved cardiac perfusion of infarct zone. Moderate and transient clinical benefits have been mostly observed in studies with intracoronary infusion or direct intramyocardial injection of BM cells. These effects are widely considered to be indirect effects of implanted cells in association with paracrine factors, cell fusion, passive ventricular remodelling, or the responses of endogenous cardiac stem cells. In contrast, evidence of cardiac regeneration characterised by differentiation of implanted stem cells into cardiomyocytes and other cardiac cell lineages, is weak or lacking. To elucidate a clear risk-benefit of this exciting therapy, future studies on the mechanisms of cardiac cell therapy will need to focus on confirming the ideal cell types in relation to dosage and timing for post-MI cardiac repair, developing more effective cell delivery techniques, and devising innovative cell tracking modalities that could unveil the fates of implanted cells such as survival, engraftment and functionality.

Keywords: bone marrow transplantation, cardiac repair, heart failure, myocardial infarction, stem cells

Singapore Med J 2009; 50(10): 935-942

INTRODUCTION

Acute myocardial infarction (AMI) as a consequence of coronary artery disease has significant detrimental effects. The affected left ventricle of patients who survived AMI may undergo negative remodelling (characterised by the replacement of necrotic myocardium with scar tissue which is made of fibroblasts and collagen) in about six months despite successful revascularisation of the infarct artery. Eventually, it deteriorates into heart failure as the left ventricle function is compromised. Post-myocardial infarction (MI) heart failure is one of the major causes of death and disability in the developed world,[1] including Singapore.[2] With a dismal five-year mortality rate of 50%~70% in symptomatic patients, the only effective lifesaving option is heart transplantation. However, this remains a difficult option in many parts of the world due to a severe shortage of donor hearts. New therapeutic strategies are needed to improve the prognosis and quality of life for patients who survived an AMI.

Stem cell therapy is a potentially new lifesaving option for post-MI heart failure. Cardiomyocyte loss associated with various forms of myocardial injuries has long been believed to be irreversible as cardiomyocytes undergo terminal differentiation after birth, withdrawing irreversibly from the cell cycle.[3-5] The recent discovery of cardiomyocyte renewal/regeneration in the human

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heart has shed new light on the treatment of post-MI heart failure.[5-8] Subsequently, preclinical studies have shown encouraging results suggesting potential benefits of stem cell-based cell therapy for post-MI cardiac repair and regeneration.[9-15]

CURRENT PROGRESS IN STEM CELL THERAPY FOR POST-MI HEART REPAIR

Encouraged by pioneer pre-clinical studies, a number of early phase clinical trials have been conducted. It is generally accepted that stem cell therapy for post-MI cardiac repair should be conducted within two weeks post-AMI before scar formation, and autologous cells are most suitable for transplantation because they obviate the need for immunosuppression. Some of the major clinical trials are listed in Table I.

INTRODUCTION OF MAJOR TRIALS

Most of the completed clinical trials were conducted in AMI patients (Table I); similar study designs were adopted, where patients aged 18–75 years of both genders (although the majority were men) were randomly recruited. Studies were conducted in Germany, Belgium, Denmark, Norway, China and the USA. In these studies, all patients had suffered recent ST-elevation MI (STEMI) and had received a successful percutaneous coronary intervention (PCI) and stenting. Moreover, most of the patients had a global left-ventricular ejection fraction (LVEF) between 30% and 45% (normal range 50%–70%) and a persistent local wall motion abnormality related to the recent infarction. Many studies were double-blinded and patients involved were randomly assigned into cell therapy and control groups (most cases as placebo controls). Additionally, several small-scale clinical studies conducted in chronic heart failure patients were also included (Table I).

As shown in Table I, all of the clinical studies exclusively involved the patients' own bone marrow (BM)-derived cells, while the major trials on AMI can be classified into two categories: (1) Trials with granulocyte-colony stimulating factor (G-CSF) mobilisation, a common procedure by mobilising endogenous BM cells (e.g. $CD34^+$ cells) by G-CSF for 5–6 consecutive days post-MI. (2) Trials with transplantation of BM-derived autologous cells. The latter was mostly done by intracoronary infusion (a route similar to the PCI procedure) that was conducted within a week post-AMI. Moreover, in two small-scale pilot studies, the transplantation was carried out by direct intramyocardial injection (epicardial injection to the border of damaged myocardium during coronary artery bypass grafting [CABG]).[16,17] In addition, catheter-based endocardial injection was adopted in a study.[18]

In those trials involving BM cell transplantation, the patients' BM was drawn and the total unfractionated mononuclear cells (MNC) were isolated by density gradient separation for immediate transplantation. MNCs were directly transplanted in most of trials, while in a few exceptions, subfractions of BM-MNCs, such as $CD133^+$ endothelial progenitor cells or $CD34^+$ haematopoietic stem cells, were further sorted out by fluorescence-activated cell sorting prior to transplantation. In a pilot study, BM mesenchymal stem cells (MSC) were isolated and expanded via a two-week culture prior to transplantation by intracoronary infusion to patients 18 days post-AMI.[19]

The endpoint analysis was carried out from one month to 18 months. Improvements in cardiac contractility such as LVEF, end-systolic volume, wall motion and suppression of left ventricle negative remodelling (reduction in infarct size and increment in velocity of infarct wall or zone, systolic wall thickening and perfusion) were measured by magnetic resonance (MR) imaging, single photon emission computed tomography (SPECT) / positron emission tomography (PET), and echocardiography.

OUTCOMES FROM MAJOR CLINICAL STUDIES

As shown in Table I, all studies appeared to be safe with a follow-up of up to 1.5 years without notable side effects as reported previously, such as coronary restenosis[20] and arrhythmia,[21] as compared to the control patients. Clinical benefits included improved cardiac contractile function and suppressed left ventricle remodelling (reduced infarct sizes, etc). Clinical benefits were reported in over half of the trials and most studies were conducted by coronary infusion/intramyocardial injection of stem cells, while the benefit of G-CSF mobilisation was less consistent. The best results appeared to be around 4–6 months post-treatment. In two studies, effects waned off 12 and 18 months post-cell therapy. Overall, clinical benefits are marginal and sometimes transient, showing a lack of durability.

DISCUSSION

The ambivalent outcomes from the current trials may be associated with several factors, including the low percentage of adult stem cells in the BM MNC, low delivery efficiency (ending up with fewer cells reaching the infracted zone), and low, if any, transdifferentiation, and finally, poor survival, engraftment and integration of the implanted cells. The marginal and sometimes transient clinical benefits appear to be significant statistically rather than clinically. Such effects have been considered as secondary effects not associated with the direct cardiogenesis of implanted cells. There is so far little evidence suggesting transdifferentiation of implanted BM-derived cells into any

Table I. Summary of major clinical trials with bone marrow-derived stem cells.

Study (Country)	No. of patients/ controls	Entity cardiac status	Cell types and dosage	Study design	Timing post-MI (days)	Other interventions	Cell delivery routes	Outcome		
								Follow-up (month)	↑ Contractility	↓ LV
FIRSTLINE-AMI[22] (Germany)	15/15	STEMI	MNC $CD34^+$	Randomised + controlled	Day 1~6	PCI & stenting	Mobilisation by G-CSF	4 & 12	↑ LVEF (++)	↑ SWT
STEMMI[23] (Denmark)	39/39	STEMI	MNC $CD34^+$	Randomised + placebo controlled	Day 1~6	PCI & stenting	Mobilisation by G-CSF	6	No effects	↑ SWT ↑ viability of infarct zone/wall
REVIVAL[24,25] (Germany)	56/58	STEMI	MNC $CD34^+$	Randomised + placebo controlled	Day 1~6	PCI & stenting	Mobilisation by G-CSF	4 & 6	No effects	No effects
G-CSF-STEMI[26] (Germany)	22/22	STEMI	MNC $CD34^+$	Randomised + placebo controlled	Day 1~5	PCI & stenting	Mobilisation by G-CSF	3	No effects	ND
BOOST[27,28] (Germany)	30/30	STEMI	MNC 2.5×10^9	Controlled	6	PCI & stenting	Intra-coronary infusion	6 18	↑ LVEF (+) ↑ Regional contractility No effects	ND
REPAIR-AMI[29,30] (Germany)	102/102	STEMI	MNC 2.4×10^8	Placebo controlled	4	PCI & stenting	Intra-coronary infusion	4	↑ LVEF (++)	↓ infarct size
TOPCARE-AMI[31-33] (Germany)	29 30	AMI AMI	MNC 2.4×10^8 CPC 1.3×10^7	Nonrandomised open-labelled Nonrandomised open-labelled	3~7	PCI & stenting	Intra-coronary infusion	4~12	↑ LVEF (++) ↑ wall motion of infarct zone	↓ infarct size
Janssens et al[34] (Belgium)	33/34	STEMI	MNC 3×10^8	Placebo controlled	1	PCI & stenting	Intra-coronary infusion	4	↑ regional systolic function	↓ infarct size ↑ viability of infarct zone
ASTAMI[35] (Norway)	50/50	STEMI*	MNC 8.7×10^7	Randomised + placebo controlled	5~8	PCI & stenting	Intra-coronary infusion	6	No effects	No effects
Chen et al[19] (China)	34/35	STEMI	MSC $48~68 \times 10^{10}$	Placebo controlled	18	PCI & stenting	Intra-coronary infusion	3 & 6	↑ LVEF (+++) ↑ regional contractility	↑ viability of infarct zone/wall
IACT[36] (Germany)	18/18	Chronic MI > 5 months	MNC	Controlled	5~102	PCI & stenting	Intra-coronary infusion	3	↑ LVEF (+++)	↓ infarct size ↑ velocity of infarct wall
Stamm et al[17] (Germany)	12	Post-MI IHD CHF	$CD133^+$ 1.5×10^6	Randomised	27 ± 31	CABG	Intra-myocardial injection (epicardial)	3 & 10	↑ LVEF (++) ↓ LVEDV	↑ perfusion in infarct zone
Patel et al[16] (USA)	10/10	Post-MI IHD CHF	MNC $CD34^+$	Controlled	Not mentioned	Off pump CABG	Intra-myocardial injection (epicardial)	1, 3, 6	↑ LVEF (++)	ND
Perin et al[18,37] (USA)	14/7	Post-MI IHD CHF	MNC $10^3/mm^2$ infarct area	Nonrandomised open-labelled	Not mentioned	PCI & stenting or CABG	Intra-myocardial injection (endoardial)	2, 4 6, 12	↑ LVEF (++) No effects	ND

*With cardiac functions well preserved

ND: not determined; G-CSF mobilisation: granulocyte-colony stimulating factor 10 μg/kg; STEMI: ST-elevated myocardial infarction; IHD: ischaemic heart disease; CHF: chronic heart failure; PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; MNC: mononuclear cells; CPC: circulating progenitor cell; LVEF: left-ventricular ejection fraction; LVEDV: left-ventricular end-diastolic volume; SWT: systolic wall thickening

Notes:

(1) LVEF, LVEDV and wall motion were determined by MR imaging, SPECT and echocardiography; ↑ LVEF(+): < 5%, ↑ LVEF(++): 5%~10%, ↑ LVEF(+++): 10%~15%. Increment compared to the controls.

(2) Infarct size and SWT were determined by MR imaging. Perfusion and viability of infarct wall/zone were determined by SPECT/PET.

Table II. Major cell types with potentials for cardiac cell therapy.

Source	Name	Potency (cardiac cell lineages)	Autologous transplantation	Tumouri-genesis	Immune-rejection	Ethical concerns
Bone marrow	MSC[19]	Multipotent (CM)	Yes	No	No	No
	CLC[53]	Multipotent (CM)	Yes	No	No	No
	C-kit$^+$ cells[54-56]	Multipotent (CM + EC + SMC + FB)	Yes	No	No	No
	EPC (CD133$^+$)[17,36]	Monopotent (EC)	Yes	No	No	No
	ELC[56,57]	Pluripotent (CM + EC + SMC + FB)	Yes	No	No	No
Fibroblast	iPS[58,59]	Pluripotent (CM + EC + SMC + FB)	Yes	Yes	No	No
Heart	CSC (SP, c-kit$^+$, Sca-1$^+$, Isl-1$^+$)[51,60,61]	Multipotent (CM + EC + SMC + FB)	Yes	No	No	No
Embryo	ESC[60,62]	Pluripotent (CM + EC + SMC + FB)	No	Yes	Yes	Yes

MSC: mesenchymal stem cells; CLC: MSC-differentiated cardiac-like cells; EPC: endothelial progenitor cells; ELC: embryonic-like cells; iPS: induced pluripotent stem (cells); CSC: cardiac stem cells; SP: side population cells; ESC: embryonic stem cells; CM: cardiomyocytes; EC: endothelial cells; SMC: smooth muscle cells; FB: fibroblasts

cardiac cell lineages *in vivo*. Accumulating evidences have attributed the clinical benefits to several effects, including: paracrine effects,[38-48] angiogenic effects,[39-48] cell fusion,[49] passive mechanical effects,[50] and endogenous responses of cardiac stem cells (CSC).[47]

FUTURE RESEARCH DIRECTIONS

Future research may focus on two aspects. The first is to understand direct mechanisms of the transplanted cells on the heart as a whole. Scientifically, these deal with such issues as the fate of implanted cells *in vivo* and their interactions with the host myocardium after transplantation. The second is to improve the evaluation of the efficacy of cardiac cell therapy in the clinical scenario by understanding cell tracking with various imaging modalities.

FUTURE LABORATORY AND PRECLINICAL RESEARCH

Studies on the mechanisms of the stem cell therapy

The post-MI myocardium is a complex environment where endogenous CSCs may participate in cardiac repair.[5,51] Transplantation of BM cells may activate endogenous CSCs for post-MI cardiac repair.[47] Thus, studies on the complicated mechanisms of stem cells therapy may lead to novel or optimised therapies targeting CSCs for their role in cardiomyogenesis and angiogenesis. For example, a cocktail of small molecules, such as of growth factors or cytokines, may be developed and injected into the affected myocardium with or without cell transplantation.[51,52]

Identification of the best cell types

The optimal goal for stem cell-based cardiac repair is to restore cardiac structure and function through regeneration of functionally-competent myocardium through cardiomyogenesis and angiogenesis. Animal studies suggest that this goal is achievable with more potent stem cells such as embryonic stem cell (ESC) or CSC (Table II). Thus, selecting better cell types might enhance the therapeutic efficacy.

Table II shows the possible sources of stem cells for cardiac repair. Overall, autologous stem cells will remain the most suitable cell type due to safety concerns (reduced risk of tumourigenesis) and immunocompatibility of the cells. Within BM-derived autologous adult stem cells, fractions of MNC may be more effective. MSCs, C-kit$^+$ BM cells and/or CD133$^+$ cells have been tested. Among them, MSCs have demonstrated promising potential in differentiating into cardiac-like cells *in vitro*,[53] and as a feasible and effective source of adult stem cells for post-MI cell therapy.[19] Embryonic-like multipotent or pluripotent adult stem cells have been recently identified in multiple tissues including BM. A recent breakthrough has shown that somatic cells, such as fibroblasts, can be reprogrammed into induced pluripotent stem (iPS) cells similar or indistinguishable from ESCs at the epigenetic and functional levels.[58,59] In future, human iPS cells may be obtained in the laboratories, and these cells could serve as ES-like autologous adult cells for cell therapy.

In most cases, getting a sufficient number of a specific cell type from the BM may require either extremely large

quantities of BM from patients, or *ex vivo* expansion of cells by culture that takes time and may mean missing the opportune window for re-introduction of the cells. This problem may be overcome by applying intramyocardial injection to deliver the cells that may require fewer cells for equal or better efficacy.

Development of optimised cell delivery modalities

In principle, effective cell therapy for injured tissue requires local delivery of cells to the proximity of the damaged tissue to avoid diffusion to other tissue/organs. Additional improvement on the efficacy of cell therapy could be achieved through optimised cell delivery procedures. At present, catheter-based stem cell delivery modalities, such as intracoronary infusion and transendocardial injection, are commonly adopted in pre-clinical studies and human trials.

Intracoronary infusion is the most common cell delivery route in clinical studies. Similar to PCI, cells delivered via a balloon catheter placed in the affected coronary artery with the inflated balloon bring temporary occlusion of the proximal section of the coronary artery to prevent back flow of the cells. Although generally considered as a safe procedure, intracoronary cell infusion is not free of potential risks. Firstly, it has been reported that an increased presence of BM or peripheral blood-derived stem cells/progenitor cells in coronary blood flow by either intracoronary infusion or G-CSF mobilisation is associated with an increased incidence of coronary events, such as coronary artery restenosis after coronary angioplasty[20] and a higher incidence of decreased coronary blood flow.[63] Secondly, it has been noted that a significant quantity of the implanted cells could home in to non-targeted organs.[63,64] This may represent a potential risk to patients. In addition, it is also an ineffective cell delivery procedure as many cells may fail to reach the infarcted myocardium.

Transendocardial injection delivers cells to small areas of abnormal heart tissue intramyocardially through an injection catheter guided by a cardiac mapping system. The procedure is regarded as safe in good hands and has been tested on animal models and on human subjects.[11,18,63,65-67] In brief, the damaged myocardium will be located, first with an electrocardial mapping system, such as the NOGA.[68] Then stem cells are injected around the border zone of the infarct myocardium via the endocardial route by an injection catheter guided by the mapping system. Transendocardial injections may improve the efficacy of cell therapy with fewer cells required. Thus, a small fraction of BM-derived cells may be used effectively for cardiac therapy. Clinical trials using transendocardial injections are currently in progress.

Development of safe and effective cell tracking modalities

Safe and effective cell tracking is necessary for a better understanding of the fate of implanted cells *in vivo* and their roles in cardiac repair. In cardiac cell therapy, safe cell tracking modalities with high sensitivity and spatial resolution are required. To date there is no satisfactory cell tracking procedures applicable to human subjects. At present, implanted cells might be directly labelled with contrast agents that have already been approved for clinical applications in combination with MR imaging, SPECT, and PET. However, direct labelling only facilitates short periods of tracking, since the labelling materials would be diluted during cell division, or diffused or degraded over time.[69] These problems could be overcome by labelling the cells with genetic manipulation. However, genetically-manipulated cells are inappropriate for clinical applications.

FUTURE CLINICAL TRIALS

Stem cell-based cell therapy may deliver considerable clinical benefits as BM cells may consistently produce many therapeutic cytokines *in situ*, thereby exerting prolonged positive paracrine effect on myocardial healing and repair. Future cell therapy trials may focus on the following aspects:

(1) To test and confirm the most beneficial subpopulations of autologous stem cells.

(2) To identify the optimal dosage and timing of cell therapeutics. The optimal cell dosage is still not clear, while the best timing of cell therapy may not be limited to the first week of AMI (Table 1). At present, more cell therapy studies in combination with CABG are being conducted (refer to: www.clinicaltrials.gov).

(3) To explain and explore the mechanisms of cell therapy in humans. Key questions, such as whether the beneficial effects of stem cells are produced by paracrine mechanisms rather than by myocardial regeneration, remain to be answered. BOOST II, a new ongoing study following BOOST I, aimed to clarify some of the issues by implanting normal cells (i.e. cells able to proliferate and secrete paracrine factors) and irradiated cells (i.e. cells unable to proliferate but still able to secrete paracrine factors) in different patients and comparing their effects.[70]

(4) To produce optimum cell delivery and homing capacity. Besides transendocardial injections for targeted cell delivery, other approaches are being tested. For example, the ongoing CELLWAVE trial aimed to determine if pre-treating the heart with ultrasonography-guided low-energy shock waves

could increase cytokine levels in the heart, which could in turn augment the homing capacity of subsequently injected cells.[70]

SPECIFIC SET-UP REQUIRED FOR CLINICAL CELL THERAPY TO ENSURE QUALITY CONTROL AND SAFETY

Despite the uncertain outcomes, research and development in stem cell applications worldwide is moving rapidly towards clinical application. At the moment, there are close to 50 ongoing clinical trials registered with ClinicalTrials.gov. Before being safely administered to patients, BM-derived cells need to be isolated and processed in the laboratory. While processing BM-MNCs takes only a few hours, the procedure for preparing BM-derived MSCs, a fraction of BM-MNCs, will take three weeks (from isolation of BM-MNC to cell expansion and differentiation). Thus, application of BM-derived cells to patients is a complicated process starting from the operating theatre, then moving to the laboratory, and finally returning to the operating theatre or cardiac catheterisation laboratory. In today's clinical practice, such a process must comply with good manufacturing practice (GMP) to reduce risks to the eventual recipients. Accordingly, cell therapy centres will likely be GMP accredited, wherein trained personnel will operate the specific facilities (clean room and cell culture laboratory facilities) following the GMP standards. Similar set-ups for haematopoietic stem cell therapy are adopted in many hospitals worldwide.

Cell processing may inadvertently expose human cells with a variety of hazards and contaminations. The major concern is zoonotic contamination, as animal-derived materials (e.g. foetal calf or bovine serum) are commonly used in cell culture. This problem could be overcome by using commercially-available serum-free media (with growth supplements). In the meantime, autologous human serum may be considered as a replacement of animal serum.[71] Finally, prior to delivery to patients, cell supernatants must be tested for infectious agents at different time points dependent on the specific protocol (e.g. Gram staining).

CONCLUSION

The optimal goal for cell-based cardiac therapy is to restore cardiac structure and function through regeneration of myocardium in the form of cardiomyogenesis and angiogenesis. This goal has not been conclusively seen in the clinical scenario. Further confirmation on the mechanism of cardiogenesis, arising from cell transplantation, will have to be conducted in the preclinical scenario in order to understand these mechanisms better. These mechanisms will then have to be proven in the clinical environment, through rigorously-conducted trials, of which cell tracking and imaging, besides markers of cardiac output, will be key components. We believe in the immense potential of cell therapy, but until substantial evidence supports the use of such therapy for the heart, we should continue to focus on the translation of laboratory-based studies to enhance our knowledge to unravel the complexity of cardiac cell therapy.

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