Review Article

Stem Cell Therapy for Acute Myocardial Infarction

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Key words:
Ventricular remodelling, ventricular function, myocyte regeneration.

Manuscript received:
September 19, 2005;
Accepted:
November 1, 2006.

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Cardiovascular diseases, especially coronary artery disease, are the leading causes of mortality and morbidity worldwide.[1] The annual cardiovascular disease deaths are estimated to be 14.3 million worldwide, of which about 70% occur in developing countries.[2] It has been reported that the prevalence of coronary artery disease in the USA reaches 6.9%, and that of myocardial infarction 3.5%.[3] An Iranian study showed a prevalence of 9.3% of symptomatic coronary artery disease in the urban population of Isfahan.[4] The MONICA (monitoring trends and determinants in cardiovascular disease) project, conducted by the World Health Organisation, monitored the trend of coronary heart disease across 37 populations in 21 countries from all four continents. The 10-year report from this project shows that the mean annual rate of coronary events in these populations is 537/100,000.[5]

Ventricular dysfunction is a common finding after myocardial infarction. During the acute phase, the contractile function is lost in the infarct area. Subsequently, there is a remodelling of the non-infarcted area causing further ventricular dysfunction.[6] This increases the mortality and morbidity in the affected patients. An international study with nine participant countries has shown that 80% of patients who die and 59% of patients who develop major complications after myocardial infarction have heart failure (HF) or left ventricular systolic dysfunction (LVSD) either on admission or during hospitalisation.[7] Ventricular dysfunction was recognised as an important prognostic predictor as early as 1967,[8] and as a result a trend has been established to increase patients' survival by improving ventricular function. In post-infarction patients with ventricular dilatation and in experimental animals, it has been shown that attenuation of dilatation decreases the rate of complications.[9-11]

As the infarcted area and ventricular remodelling are causes of LVSD, the major goal for prevention and/or reversal of this process would be the enhancement of regeneration of cardiac myocytes, as well as the stimulation of neovascularisation within the infarct area.[12] The current established strategies to minimise necrosis and subsequent LVSD and HF are angioplasty and fibrinolysis during the acute phase of myocardial infarction. Late revascularisation procedures also help to salvage myocardium in the areas that contain a minimal number of viable, reversibly injured myocytes (areas of hibernating myocardium).[13] However, these procedures cannot repair or replace completely damaged myocardium. Although human cardiomyocytes are reported to proliferate and contribute to the increase in muscle mass of the myocardium after infarction,[14] their capacity for regeneration, mitigation of the adverse effects of ventricular remodelling, and contribution to cardiac function is limited.[15] Recently, insights into

stem cell plasticity have opened up new perspectives for regenerating the infarcted heart and a wide range of stem/progenitor cell types have been used for cardiac cell therapy.

Three different approaches are possible for cardiac cell therapy: 1) transplantation of stem cells into the infarcted area;[16] 2) mobilisation of bone marrow stem cells at the site of injury with the use of cytokines and/or stem-cell factor;[17] and 3) administration of local treatment with growth factors, such as insulin-like[18] and hepatocyte growth factors,[19,20] which induce the differentiation of cardiac progenitor cells into cardiomyocytes.[15] Generally speaking, stem cells are believed to improve myocardial function by increasing or preserving the number of viable cardiomyocytes, improving the vascular supply, and augmenting the contractile function of the injured myocardium.[21]

Stem cells

Although it is difficult to find a universally acceptable definition of the term "stem cell" that serves to distinguish it from non-stem cells, certain attributes can be assigned.[22] The current most widely used definition of stem cells is: clonogenic cells capable of both self-renewal and multilineage differentiation.[23] In fact, a stem cell is a special kind of cell that has a unique capacity to renew itself and to give rise to specific cell types. Although most cells of the body, such as muscle cells, are committed to fulfilling a particular function, a stem cell is uncommitted until it receives a signal to develop into a specialised cell.[24]

Stem cells can be obtained from embryonic, foetal and adult tissues. Based on their differentiation potential, stem cells can be: i) pluripotent, meaning that they can individually give rise to all types of cells that develop from the germ layers (endoderm, mesoderm and ectoderm) and germ cells;[25] ii) totipotent, cells that have the capability of pluripotent cells plus the ability to give rise to placental tissue; iii) unipotent, can give rise to only one type of differentiated cell; and iv) multipotent, a state between unipotent and pluripotent.[26]

Embryonic stem cells

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the blastocyst, an early embryonic stage.[27] Their derivation was first reported in 1981 from mice,[28,29] and in 1998 from humans.[30] It has been known for many years that pluripotent embryonic stem cells can proliferate indefinitely *in vitro* and are able to differentiate into derivatives of all three germ layers.[31] Human ES cells can proliferate for 300 population doublings.[32] Therefore, when established as a cell line, they would be marketable and easily available as a therapeutic cell source. So far, the therapeutic potential of cells derived from differentiating ES cells has been investigated in a number of studies. When undifferentiated ES cells were transplanted into the infarcted heart, they differentiated into functional cardiac myocytes and improved cardiac function in both mice[33] and rats.[34] Murine ES cell-derived cardiomyocytes survived upon transplantation to the heart of dystrophic mice[35] and mice with cardiac infarction,[36] and improved cardiac function in the latter. They also survived when transplanted into sites other than the heart.[37]

Several issues must be resolved before we can consider the application of ES cells in clinical setting. There is a strong worldwide ethical debate about the ethics of using ES cells for therapeutic purposes.[38,39] If a therapeutic modality develops using human ES cells, there is a potential for these ethical issues to prevent the spread of this modality to certain populations. Therefore, it seems more reasonable to concentrate scientific efforts on modalities which, when developed, can be applied to all populations without dispute. Tumourigenicity of ES cells after transplantation is a very important issue that should be properly addressed before starting ES cell transplantation clinical trials. It has been shown that these cells have the potential to induce tumour formation after transplantation.[40] The last concern is the fact that these cells are allogeneic and express high levels of MHC-I proteins and thus may be rejected on transplantation.[41] In view of these issues, ES cells cannot be considered as the first choice in a clinical trial experiment at present.

Foetal stem cells

Primitive cell types in the foetus that eventually develop into the various organs of the body are called foetal stem cells.[42] So far, in a limited number of studies, foetal cardiomyocytes have been transplanted into animal models of myocardial infarction; and showed promising results.[43-46] However, the safety of transplantation of foetal stem cells has yet not been adequately addressed. Meanwhile, there are significant ethical issues in connection with the clinical application of foetal stem cells. Therefore, it seems that this cell source is far from clinical application at present.

Although not part of the foetus, human umbilical

vein endothelial cells (HUVEC) have also received attention as a possible cell source. In an animal model study, they have been transplanted into the infarcted heart and improved cardiac function through increased neovascularisation.[47] However, experiments with HUVEC are still in their infancy, and these cells cannot yet be employed in a clinical setting.

Human umbilical cord nucleated cells have also been used as a potential source for cell therapy in animal models. They improved cardiac function by increased neovascularisation.[48-50] Nevertheless, their safety as a cell source needs to be confirmed in animal studies.

Adult stem cells

Adult stem cells are undifferentiated cells present in differentiated, specialised tissue. Basically, these cells renew themselves and become specialised to yield all of the mature cell types of the tissue from which they originated. Not long ago, it was shown that adult stem cells can develop not only into the specialised phenotypes of their tissue of origin but also into cell types of another tissue derived either from the same embryonic germ layer or from a different one. This is called plasticity.[24] For example, it has been shown that bone marrow stem cells can differentiate into tissue that is mesodermal,[51-53] ectodermal,[54] or endodermal.[55] Although not synonymous, the terms "stem cells" and "progenitor cells" are used interchangeably in the literature dealing with bone marrow and peripheral blood stem cells.

Skeletal myoblasts

Skeletal myoblasts are also called satellite cells. They are present in the basal lamina of adult muscle fibres. They are committed stem cells and can only differentiate into muscle cells.[56] Another important feature of these cells is their high resistance to ischaemia.[57] Experimental animal studies have shown that transplanted myoblasts after myocardial infarction are engrafted and lead to improvement of cardiac function.[58-61] However, these cells differentiate into mature skeletal muscle within the injured myocardium and do not express cardiac-specific genes after grafting into the heart.[61] This means they do not establish cardiomyocyte-specific intercellular junctions with cardiomyocytes, and theoretically do not couple with cardiomyocytes electromechanically. But *in vitro* studies have shown that skeletal myoblast grafts beat synchronously with cardiomyocytes.[60,62]

Early arrhythmogenicity is another concern after skeletal myoblast transplantation.[63,64] One theory states that the inability of the grafted myoblast to form junctions with cardiomyocytes produces re-entry arrhythmias. However, if that was the case, the arrhythmogenicity would be late, when myoblasts are differentiated.[56] In a phase I clinical trial, the arrhythmogenicity was successfully managed with prophylactic amiodarone infusion before and during the procedure, and amiodarone was discontinued after 6 weeks.[64]

So far, phase I clinical trials have been performed by transplantation of autologous skeletal myoblasts to the infarcted myocardium.[63-65] They have shown improved cardiac function after transplantation. There is also histological evidence in human subjects that upon transplantation, skeletal myoblasts survive and form viable grafts in heavily scarred myocardial tissue.[66] Currently, phase II clinical studies are in progress, evaluating the efficacy of autologous myoblast transplantation performed at the time of CABG.[64]

Bone marrow and peripheral blood stem cells

Bone marrow contains several subpopulations of stem cells of which haematopoietic stem cells (HSCs), endothelial progenitor cells, and mesenchymal stem cells have received much attention. Low levels of HSCs move from bone marrow to peripheral blood under normal conditions.[67] It is possible to harvest HSCs from peripheral blood in sufficient quantities as an alternative to bone marrow transplantation.[68,69] Endothelial progenitor cells can also be found in peripheral blood.[70]

Haematopoietic stem cells

Haematopoietic stem cells do not express a number of surface markers that are expressed by mature blood cells. Lack of expression of these lineage (lin) markers can be used for selection of these cells. Examples of the markers commonly used to isolate human lin– cells are glycophorin A, CD2, CD3, CD4, CD8, CD14, CD15, CD16, CD19, CD20, CD56, and CD66b.[71] Selection of lin– cells typically gives a 20- to 500-fold enrichment of HSCs, depending on the combination of lin markers used.[72] However, CD34 is considered as the universal marker for HSCs,[73] and positive selection for this marker gives a 25- to100-fold enrichment of HSCs.[71] It has been shown that not all HSCs are positive for CD34.[74] CD133 (formerly known as AC133) is another marker for HSCs. About 80% of the CD34+ cells are positive for CD133, while less than 20% of CD133+ cells are negative for CD34.[75] It has been suggested that CD133 protein is a more immature HSC marker.[76] Other mar-

kers for human HSCs are CDCP1,[77] C-KIT (also called CD117),[78] and VEGFR-2 (also called KDR).[71]

The point to be remembered is that all white blood cells, red blood cells and platelet aggregates express CD34. When white blood cells are the targets of any purification, expression of CD45, which is only expressed on white blood cells, is taken into consideration.[24]

Side population is a fraction of bone marrow highly enriched with HSCs. They can be isolated by flow cytometry on the basis that they actively exclude Hoechst 33352 dye.[79] Their phenotype is described as CD34–/low, c-Kit+, Sca-1+.[80]

Endothelial progenitor cells

Endothelial progenitor cells (EPCs) are believed to share a common putative precursor —haemangioblast— with HSCs.[81,82] However, controversy exists with respect to their origin.[83] They are bone-marrow derived cells in the peripheral circulation; they have the capability to differentiate to endothelial cells,[70] and are recruited to foci of neovascularisation such as ischaemic myocardium.[84] In bone marrow they are characterised by a CD133+/CD34+/ VEGFR-2+ phenotype. In the peripheral blood of adults, more mature EPCs are found, which do not express the CD133 marker and have a phenotype of CD34+/VEGFR-2+/ CD31+/ VE-cadherin+.[70] Mature endothelial cells show a high expression of VEGFR-2, VE-cadherin, and von Willebrand factor.[70]

EPCs are prepared by isolation of: 1) CD34+ mononuclear cells from bone marrow,[85] peripheral blood,[81] and cord blood;[86] 2) CD133+ mononuclear cells from bone marrow,[87] cord blood,[88] and granulocyte colony-stimulating factor (G-CSF)-mobilised peripheral blood;[89] 3) nucleated cells in peripheral blood that form adherent cultures.[90] The isolated cells are then cultured *in vitro* on fibronectin-coated flasks in the presence of a number of specific growth factors.[87,91]

It has been shown that in patients with acute myocardial infarction, the CD34+ mononuclear cell population in the peripheral blood stem cell pool increases.[92] They are also found in the umbilical vein blood and known as cord blood stem cells.[86]

Bone marrow mesenchymal stem cells

Bone marrow mesenchymal stem cells (MSCs) are also known as marrow stromal cells, mesenchymal stromal cells, and mesenchymal progenitor cells.[93] They are a fraction of bone marrow nucleated cells that form adherent cultures.[94] There are no markers which specifically and uniquely identify MSCs, and they are therefore defined by their immunophenotypic profile (see Roberts, 2004[95]) and by their characteristic morphology. MSCs are fibroblastic-like cells and do not express haematopoietic markers such as CD14, CD34, CD45 or CD133, or the endothelial markers von Willebrand factor and P-selectin.[96] It has been suggested that these cells are uniformly positive for CD90, CD105, and CD166.[93] See Pittenger and Marshak, 2001,[96] and Pittenger and Martin, 2004,[97] for a comprehensive list of surface molecules on human MSCs, and see Alhadlaq et al, 2004,[98] for isolation techniques. In an animal model study, it has been shown that MSCs can be mobilised after acute myocardial infarction and differentiate into cardiomyocytes.[99]

These cells can be induced to differentiate into mesenchymal lineages such as osteoblasts, chondrocytes[55] and cardiomyocytes.[100,101] The most exciting feature of MSCs is the possibility of the allogeneic use of these cells without immunosuppression, because they are poor antigen-presenting cells and do not express major histocompatibility complex (MHC) class II or co-stimulatory molecules (see Bacigalupo, 2004,[102] for review).

Animal model studies

A few animal model studies have shown that mobilised HSCs,[103] transplanted HSCs[53] and side population[80] after myocardial infarction can differentiate into cardiomyocytes. Improvement in cardiac function has also been reported.[104] But other studies have not confirmed these results and failed to show any differentiation of these cells into cardiomyocytes.[104-107]

Animal model studies showed that transplanted EPCs improve cardiac function after myocardial infarction,[52,84,108] lead to better preservation of capillary density,[84,108] and incorporate into sites of neovascularisation.[81,84] In a key observation, it has been shown that cultured bone marrow-derived CD34+ cells secrete vascular endothelial growth factor (VEGF), and upon transplantation to the primate model of myocardial infarction increase the VEGF level in the myocardium.[109] This raises the possibility that increased neovascularisation might be due to paracrine effects of transplanted cells.[110]

It should be pointed out that angiogenic growth factors such as VEGF and fibroblast growth factors (FGFs) are already undergoing clinical trial for coronary artery disease. But so far, the overall results of these trials have not been promising (see Annex and Simons, 2005,[111]

and Freedman et al, 2002,[51] for review). When comparing angiogenic growth factor therapy with stem cell therapy for coronary artery disease, one must consider the evidence that ischaemia upregulates a number of different growth factors, for example VEGF, FGF, and epidermal growth factor, leading to both local angiogenesis[112] and the mobilisation of stem/progenitor cells from bone marrow.[92] Stem cell therapy augments the effects of mobilised bone marrow stem cells, which could be more extensive than the effects of administering an angiogenic growth factor alone.[93]

Human bone marrow-derived CD133+ cells were injected into the myocardial scar of rats 10 days after induction of myocardial infarction.[113] The animals were followed for one month. Cardiac function was improved. However, the cells could not be tracked in five hearts and only a few cells could be detected in the remaining eight. When the benefit of CD133+ implantation was compared to that of skeletal myoblasts, no superiority was found.

In animal models it has been shown that transplanted MSCs transdifferentiate into cardiomyocytes[114,115] and endothelial cells,[114,116] and contribute to the improvement of cardiac function.[114-116] As with the observation made with cultured bone marrow-derived CD34+, it has been shown that transplantation of MSCs after myocardial infarction increases the VEGF content of the heart and hence vascular density and cardiac function.[117] In an experimental study on dogs, it was shown that intracoronary injection of MSCs leads to myocardial microinfarction.[118] It has also been shown that the size of injected cells was about two-fold larger than that of freshly prepared nucleated bone marrow cells.[118]

Unfractioned bone marrow nucleated cells as a source of EPCs and MSCs,[119-121] and unfractioned peripheral blood nucleated cells as a source of EPCs[122] have been found to contribute to the neoangiogenesis of ischaemic myocardium. Unfractioned bone marrow cells can also form parts of regenerated cardiomyocytes as well.[123] Furthermore, it has been shown that fusion can occur between a rare population of bone marrow-derived mononuclear cells and cardiomyocytes.[107] Improvement of cardiac function by implantation of marrow mononuclear cells[124] has also been reported. The therapeutic effect of peripheral blood unfractioned nucleated cells was not confirmed in one report.[124]

Clinical trials

Mesenchymal stem cells

A randomised controlled clinical trial of autologous mesenchymal bone marrow stem cells transplantation investigated 69 patients who underwent percutaneous coronary intervention (PCI) within 12 hours after the onset of acute myocardial infarction.[125] Bone marrows were aspirated on day 8 after PCI; nucleated cells were isolated by density gradient centrifugation and cultured for 10 days. The target coronary artery was occluded for 2 minutes before injection of the cell suspension to block the anterior blood flow. Six ml of MSC suspension containing $8\text{-}10 \times 10^9$ cells/ml were injected into the artery, and the patients were followed up for 3 months. This trial, which is listed in the Cochrane Library evidence-based medicine database, concluded that the protocol was safe and led to improvement of cardiac function. However, there are concerns with regard to the bone marrow MSC isolation protocol employed in this trial. As the cells were not characterised, it is not clear if they were MSCs or just cultured bone marrow nucleated cells.[126]

Endothelial progenitor cells

Two sets of non-randomised clinical trials have been published by the same team, and involve endothelial progenitor cells derived from peripheral blood (Assmus et al, 2002,[12] and Britten et al, 2003[127]). The study was named Transplantation Of Progenitor Cells And Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Nucleated cells were isolated from peripheral blood by density gradient centrifugation. The cells were cultured on fibronectin-coated culture surfaces and the specialty culture medium was supplemented with VEGF, atorvastatin and 20% patient's serum. After three days, the cells were harvested and characterised by Dil-acetylated LDL uptake and positive staining with lectin, and expression of VEGFR-2 (KDR), endoglin (CD105), von Willebrand factor, platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31), and VE-Cadherin or CD146. More than 90% of cells showed endothelial characteristics. A mean of $10 \pm 7 \times 10^6$ (Assmus' series) and $13 \pm 12 \times 10^6$ (Britten's series) cells were injected in a suspension volume of 10 ml for each patient. The total volume was infused in 3 aliquots of 3.3 ml, and during infusion the blood flow was completely blocked for 3 minutes, interrupted by 3 minutes of reflow. In Assmus' controlled set 10 patients were reported who underwent cell transplantation 4.3 ± 1.5 days after acute myocardial infarction. A stent had been implanted in all these patients on the day of diagnosis of acute myocardial infarction.[12] In Britten's set 13 patients were

reported (some of them were also reported in the first set). They underwent the same procedure 4.7 ± 1.7 days after acute myocardial infarction.[127] The patients were followed for 4 months. There were no deaths and none of them developed any malignant arrhythmias. Therefore, the procedure was considered safe and feasible. Transplantation of EPCs decreased infarct size, improved cardiac function and increased coronary blood flow reserve in the infarct artery. They also showed that the migratory capacity of the infused cells is a major determinant of infarct remodelling.[127] It should be noted that both publications are listed in the Cochrane Library evidence-based medicine database.

CD133+ and CD34+ cells

Two non-randomised, non-controlled phase I clinical trials have been performed with purified bone marrow CD133+ cells. The cells were isolated by magnetic cell separator from nucleated fraction of bone marrow aspirate.

In the first clinical trial,[128] the CD45negative subpopulation was used for implantation. CD45 is a panhaematopoietic marker expressed on all white blood cells, and its absence of expression implies endothelial progenitor origin of the purified cells. A total of 1.23×10^6 to 3.37×10^6 nucleated cells with a CD133+ cell purity of 53-89% were injected in 6 patients during coronary artery bypass grafting (CABG). The injections were performed with a hypodermic needle along the infarct zone. Ten injections of 0.2 ml were performed for each patient. Patients were followed for 3-9 months. Apart from early complications, which could not be definitely attributed to either surgery or cell therapy, no other complications were found. Global left ventricular function was enhanced in four patients, and infarct tissue perfusion improved strikingly in five patients.

In the second clinical trial,[129] 5 patients with end-stage coronary artery disease underwent intramyocardial injection during transmyocardial laser revascularisation (TLMR) and CABG. Following standard CABG surgery, laser channels were shot in predefined areas within the hibernating myocardium. Subsequently, between $1.9\text{-}9.7 \times 10^6$ total nucleated cells with a CD133+ cell purity of 78-97% were injected in a predefined pattern around the laser channels. Follow up of two cases showed improvement of wall motion at the sites of stem cell transplantation.[130]

An ongoing phase I randomised, double-blind, placebo controlled clinical trial is under way at Caritas St. Elizabeth's Medical Centre, Boston, USA, to determine the safety of various doses of autologous CD34+ cells for cell therapy in patients with myocardial ischaemia. More details can be found on the Current Controlled Trials web site (www.controlled-trials.com).

Mobilised progenitor cells

An ongoing randomised, controlled, clinical trial, the ROT FRONT trial,[131] was started in order to elucidate the effects of mobilisation of marrow progenitor cells by G-CSF in patients with NYHA class II-IV chronic heart failure due to ischaemic heart disease, zones of nonviable myocardium and left ventricular ejection fraction <40%. It is also intended to assess the safety of the addition of G-CSF to standard therapy with ACE inhibitors and beta-blockers. So far, 5 patients and 1 control have been included in this trial. The control patient died in week 11 of the study. One young patient (48 years old) showed a 12-fold increase in white blood cell count, with appearance of myelocytes and myeloblasts accompanied by improved cardiac function. Patients older than 60 years old showed just 6-8 fold increases in leukocyte count and their cardiac function has not improved. Although the results of this study are still premature, it seems that mobilisation of marrow progenitor cells has the potential to improve cardiac function. This study has been listed in the Cochrane Library evidence-based medicine database.

In another study conducted by Hill and coworkers,[132] G-CSF was administered to 12 patients with chronic myocardial ischaemia, who had intractable angina and whose coronary lesions were not appropriate for further revascularisation. Two myocardial infarctions, one of them leading to death, have been reported in this series. G-CSF has also been administered by Kang et al[133] to 3 patients with myocardial infarctions but with relatively stable symptoms, 4 days before PCI, in a randomised, controlled study listed in an evidence-based medicine database. In contrast to Hill's series, there was no peri-procedural serious adverse reaction, but at 6 months' follow up no improvement was observed in left ventricular ejection fraction. Two of the 3 patients showed restenosis of the stent and the trial has been stopped due to the high restenosis rate (see below under "unfractioned peripheral blood nucleated cells" for more information about this trial). It has been suggested that granulocyte colony-stimulating fac-

tor may promote in-stent restenosis by enhancing neutrophil recruitment at sites of tissue injury.[134,135]

In a controlled study involving 16 patients with coronary artery disease, it has been shown that G-CSF increases EPCs in the peripheral circulation. It also increases expression of the chemokine receptor CXCR-4 on CD133+ cells. This receptor is important for homing of EPCs to ischaemic tissues.[136] In this study no measurements were performed with regard to the cardiac function and coronary blood flow in these patients.

Based on the observations that statin therapy in patients with coronary artery disease improves vasomotor response to endothelium-dependent agonists[137] and enhances coronary blood flow,[138] and that in normocholesterolaemic animals statins increase angiogenesis through modulation of the Akt signalling pathway and an increase in nitric oxide (NO) production by the endothelial type NO synthase (eNOS),[139] a study was designed to investigate the possibility of mobilisation of endothelial progenitor cells by statins as another mechanism for the angiogenic effect of these drugs. It has been shown that in patients with stable coronary artery disease, statins increase circulating endothelial progenitor cells and increase their migratory capacity.[140] This study is listed in the Cochrane Library.

Unfractioned peripheral blood nucleated cells

A randomised, controlled study performed by Kang et al,[133] part of which is mentioned above under "mobilised progenitor cells", investigated the effects of intracoronary infusion of peripheral blood stem-cells mobilised with G-CSF on the cardiac function of patients with recent myocardial infarction who underwent PCI. Patients received daily injections of G-CSF for 4 days before PCI. On the day of PCI, nucleated cells were isolated from peripheral blood by an automatic apheresis system. Seven patients received 1×10^9 unfractioned nucleated cells, with a lowest CD34+ cell yield of 0.7%, by intracoronary infusion. To minimise the risk of the "no reflow phenomenon", the patients received nicorandil and nitroglycerine by coronary guiding catheter, and achieved an activated clotting time of more than 250 s with an intravenous bolus infusion of heparin before intracoronary infusion. Three patients in the control group received G-CSF alone and one patient received nothing. No peri-procedural serious adverse reactions were noted. Six months follow up showed that cell infusion significantly improved exercise capacity, myocardial perfusion and systolic function. However, there was a high rate of in-stent restenosis (5 out of 7 cell infusion group, and 2 out of 3 G-CSF-only group). Therefore, the trial was stopped. As restenosis was observed in the G-CSF-only group as well, its attribution to G-CSF seems rational. This study has been listed in the evidence-based medicine database.

Unfractioned bone marrow nucleated cells

The trials in this category are more numerous than in others. Out of eight trials that will be mentioned here, five[12,16,127,135,141] have been listed in the Cochrane Library. Nucleated cells were isolated from bone marrow with an automatic apheresis system in one series,[142] and with density gradient centrifugation in others.

The non-randomised, non-controlled clinical trial conducted by Hamano et al,[142] recruited 5 patients who underwent coronary artery bypass grafting (CABG) and had at least one ischaemic area unsuitable for the traditional treatments of percutaneous transluminal coronary angioplasty or bypass grafting to the stenotic coronary artery. In each patient, after completion of CABG, the cells were injected into the area of ischaemic myocardium where there was no graft. Each patient received 30-220 $\times 10^7$ cells in 6-22 injections. The injection volume was 0.1 ml (5×10^7 to 1×10^8 cells/point) and injections were spaced 1 cm apart, using a 1 ml syringe and a 26-gauge needle. All were followed up for at least 1 year. No serious complications were reported. Postoperative cardiac scintigraphy showed improvement in coronary perfusion in 3 out of 5 patients. The authors concluded that cell therapy can be a viable option for ungraftable areas of myocardium.

Strauer et al[6] made an overnight culture of the isolated nucleated cells in Teflon bags with a commercial mononuclear culture medium. It was a controlled, non-randomised trial in which 10 patients received the cell therapy and 10 patients served as controls. All patients underwent angioplasty >4 hours (with a mean of 12 ± 10 hours) after the start of the infarct pain. Then, five to ten days after the onset of acute pain (i.e. during the post-infarction period), patients underwent a second percutaneous transluminal coronary angioplasty. The procedure was performed 6 to 7 times for 2 to 4 minutes each. During this time, intracoronary cell transplantation via the balloon catheter was carried out, using 6 to 7 fractional high-pressure infusions of 2 to 3 ml cell suspension, each of which contained 1.5 to 4×10^6 nucleated cells. Each patient received $2.8 \pm 2.3 \times 10^7$ cells. Nucleated cell

suspension consisted of 0.65 ± 0.4% AC133+ cells and 2.1 ± 0.28% CD34+ cells. No serious complication was reported. After 3 months of follow up, cell therapy led to a decrease in the infarct region, an increase in the infarction wall movement velocity, as well as improvement in stroke volume index, left ventricular end-systolic volume and contractility, and myocardial perfusion of the infarct region.

In the two sets of non-randomised clinical trials performed under the name of TOPCARE-AMI and mentioned above, a few patients received unfractioned bone marrow nucleated cells. Nine patients in Assmus' series received 245 ± 72 × 10^6 nucleated cells (with a mean value of 7.35 ± 7.31 × 10^6 CD34+/CD45+ cells), while 16 patients in Britten's series received 238 ± 79 × 10^6 nucleated cells (with a mean value of 5.5 ± 2.8 × 10^6 CD34+/CD45+ cells and 0.7 ± 0.4 × 10^6 CD133+ cells). Some of the patients in Britten's series were also reported in Assmus's series. The cell infusion method and patient assessment were the same as described above. The patients were followed for 4 months and no serious complication related to cell therapy was reported. Transplanted cells decreased infarct size, improved cardiac function and increased coronary blood flow reserve in the infarct artery.

Tse et al[143] performed a non-randomised, non-controlled clinical trial on 8 patients with stable angina refractory to maximum medical therapy. The ischaemic regions of myocardium were identified by electromechanical mapping. Patients received nucleated cells by direct injections into the ischaemic myocardium using a percutaneous catheter. The cell suspension contained 3.2% ± 2.4% CD34+ cells, 7.6% ± 3.5% CD3+ T cells, 43.7% ± 15.9% CD11b+ D15+ granulocyte precursor cells, and 117 ± 67.4 granulocyte-monocyte colony-forming units (CFU-GM) per 10^5 cells. Each patient received 1.2-1.6 × 10^7 nucleated cells (personal communication with Dr. Tse). No serious complication was reported and after 3 months of follow up patients had fewer episodes of angina. It has also been shown that there was improvement in myocardial perfusion and segmental contractility in the ischaemic region.

As in the previous series, the ischaemic hibernating myocardial areas were identified by electromechanical mapping and bone marrow cells were injected intramyocardially in a trial published by Perin et al.[141] This was a non-randomised controlled trial involving 14 patients and 7 controls. Each patient received 15 transendocardial injections, 0.2 ml each, using a percutaneous catheter. Every patient received a mean of 25.5 ± 6.3 × 10^6 nucleated cells. The cells were characterised as early haematopoietic progenitors (CD45low/CD34+/HLA-DR–) 0.1% ± 0.1%, haematopoietic progenitor cells (CD45low/CD34+) 2.4% ± 1.3%, CD4+ T cells 28.4% ± 10.8%, CD8+ T cells 14.9% ± 5.9%, B cells 1.9% ± 1.0%, monocytes 10.0% ± 4.0%, NK cells 1.2% ± 0.5%. Functional assays were also performed, showing that each patient received 0.2 ± 0.2 × 10^3 fibroblast colony-forming units and 16.4 ± 18.5 × 10^3 granulocyte-macrophage colony-forming units. Patients were followed for 4 months. One patient in the treatment group died 14 weeks after therapy, probably of sudden cardiac death. One patient had an early episode of pulmonary oedema. No other complications were reported and the procedure was considered relatively safe. Patients in the treatment group showed an improvement in global left ventricular function and mechanical improvements of the injected segments.

Ten patients with severe symptomatic chronic myocardial ischaemia not amenable to conventional revascularisation were entered into a non-controlled, non-randomised clinical trial by Fuchs et al.[144] Each patient received 2.4 ml of cell suspension containing 32.6 ± 27.5 × 10^6 nucleated cells with the following subfractions: PMNs 74.6% ± 6.5%, lymphocytes 19.3% ± 8.1%, monocytes 3.5% ± 1.0%, megakaryocytes 2.6% ± 2.3%, CD34+ 2.6% ± 1.6% (of which 47.9% ± 15.1% co-expressed CD45). 85% ± 14% of CD34+/CD45+ cells co-expressed CD117. The myocardial ischaemic territories were identified by electromechanical mapping, and each patient received 12 injections of 0.2 ml cell suspensions in pre-defined ischaemic areas by percutaneous catheter-based transendocardial injections. Apart from ventricular premature beats at the time of injections and admission of two patients for recurrent chest pain, no other complications were reported. At three months' follow up, angina symptoms had improved in 8 patients. There was improvement in the stress-induced ischaemia occurring within the injected territories, but there was no change in ejection fraction.

In the BOOST randomised controlled clinical trial by Wollert et al,[135] 60 patients were randomised to receive bone marrow cells (n=30) or serve as controls (n=30). Patients with a first ST-segment elevation myocardial infarction who were admitted within 5 days and had a successful PCI with stent implantation were entered into this trial. Patients underwent bone marrow harvest 5.7 ± 1.2 days after onset of the

symptoms, and 6-8 hours later, during a second PCI, each patient in the treatment group received 24.6 $\pm$ 9.4 $\times 10^8$ nucleated cells containing 9.5 $\pm$ 6.3 $\times 10^6$ CD34+ cells and 3.6 $\pm$ 3.4 $\times 10^6$ haematopoietic colony forming cells. The cells were infused in the infarct-related artery. Patients were followed for 6 months. No complication related to cell transfer was reported. Cell transfer increased the global left ventricular ejection fraction.

The above data show that bone marrow-derived stem cell therapy improves cardiac function after acute myocardial infarction. Also, the data show the feasibility and safety of this approach. However, further studies are needed to determine the optimal dose, route of delivery, time of delivery after acute myocardial infarction, and contraindications to this therapy.

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