Introduction

Despite impressive improvement in the diagnosis and management of acute myocardial infarction (AMI), this condition remains the leading cause of heart failure (HF) and continues to be a major public health concern in the industrialized world. AMI is still a fatal event in approximately one third of patients, being the major cause of death in men after 40 and in women after 65 years of age. Encouraging results have been obtained in experimental ischemic and non-ischemic heart disease that show sustained cell survival after transplantation, integration into the host myocardium, and functional improvement of diseased hearts. Furthermore, preliminary data obtained in patients with acute myocardial infarction, suggest that the observation obtained in the experimental animal may be transferred to the clinical arena in the near future. These observations fueled an exciting period of discovery and high expectations followed by controversies that need to be addressed before this strategy can be added to the therapeutic options for patients with heart disease.

Key words: Heart failure; Myocardial regeneration; Stem cells.

Cell transplantation: a novel perspective in the treatment of heart failure

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Stem cell transplantation has been proposed as a novel experimental strategy to treat heart diseases, such as acute myocardial infarction and heart failure. The beneficial effects of transplanted cells may include active contribution to contractile function, passive improvement of cardiac mechanics, induction of neoangiogenesis or other indirect influences on the biology of the heart. Several cell types have been used for cardiac transplantation. These include embryonic stem cells, bone marrow stem cells, and skeletal myoblasts. Encouraging results have been obtained in experimental ischemic and non-ischemic heart disease that show sustained cell survival after transplantation, integration into the host myocardium, and functional improvement of diseased hearts. Furthermore, preliminary data, obtained in patients with acute myocardial infarction, suggest that the observation obtained in the experimental animal may be transferred to the clinical arena in the near future. These observations fueled an exciting period of discovery and high expectations followed by controversies that need to be addressed before this strategy can be added to the therapeutic options for patients with heart disease.

on the potential role of totipotent cells (stem cells) as a source for repairing damaged myocardium. These investigations are the focus of the present review.

General considerations

Sudden occlusion of a major coronary artery leads to the rapid death of cardiac myocytes and vascular structures unless myocardial perfusion is rapidly restored. The infarct area is centrally necrosed, non-viable and scarred; usually, minimal residual perfusion is preserved especially in the epicardial layers. Depending on the initial size of infarction, structural remodeling takes place. Early reperfusion by either thrombolysis or mechanical recanalization can actually only reduce the infarct area, but complete prevention of necrosis is not possible.

Until a few years ago, cardiac myocytes were thought to be terminally differentiated cells and were often compared to neurons for their inability to regenerate and repair damaged myocardium. Thus, in the adult heart under both physiological and pathological conditions myocyte growth was believed to be restricted to cellular hypertrophy. Evidence now is accumulating that suggests that this old paradigm has probably to be changed. Indeed, myocyte replication has been demonstrated both in normal subjects and in the failing human heart where this form of cell growth tends to compensate for the exhaustion of myocyte hypertrophy. Moreover, after AMI, the abrupt increase in the need for growth causes more myocytes to reenter the mitotic cycle than during chronic HF. Evidence is accumulating that most of the colonization by host cells occurs very soon after transplantation, probably stimulated by the diffuse ischemic damage sustained by the heart. In a relatively short time (median 53 days), host cells were able to generate almost one fifth of all of the myocytes and vascular cells in the graft. The finding that, in one patient who died 4 days after transplantation, many myocytes and arterioles of host origin were fully mature and indistinguishable from the donor cells, gives an idea of how quickly the myocardial milieu can instruct the new arrivals to develop the proper phenotype. What is not clear from the data presented is the functional significance of the newly formed cardiac cells, that is whether they contribute either to the contractile performance or to the survival of the allograft.

Although numerous issues remain to be addressed, the discovery that the transplanted heart is colonized by cells with high capacity for growth and with the ability to differentiate into multiple cell types suggests that myocardial regeneration and cardiac repair may be feasible. Thus, resident cardiac stem cells in vivo may differentiate into myocytes in normal and diseased hearts. These cells are not confined to restricted regions of the heart; they migrate where they are needed.

Experimental models

Using stem cells to repair the heart raises many more questions than it answers and, more importantly, outlines some of the major hurdles spoiling this promising field. First, is there sufficient scientific evidence from animal and human data to support the concept of therapeutic myocardial regeneration? If so, what are the additional preclinical investigations necessary before human trials are undertaken? Second, if preclinical studies justify human investigation, how should these trials be performed with regard to patient selection, nature of stem cells to use, type of delivery system, as well as clinical and functional endpoints? Finally, what are the ethical and safety issues to consider when engaging in human stem cell studies?

In fact, stem cell implants have been shown to exhibit regenerative properties in several damaged organs, including irreversibly injured, ischemic myocardium. The classification of stem cells has also evolved. To simplify the origin of pluripotent cells, we may classify them into three main sources: embryonic stem cells, bone marrow cells (BMCs), and satellite cells or myoblasts.

Embryonic stem cells. Embryonic stem cells originate from the inner mass of the blastocyst or from primordial germ cells and can be propagated in vitro for a virtually unlimited time to the stage of totipotent ability, thus raising the possibility of being useful for the regeneration of every tissue and organ in the human
The results of investigations in this area confirm that, depending on the specific microenvironment, embryonic stem cells may mature and acquire all the characteristics of the target tissue. Because embryonic stem cells tend to imitate the physiological as well as the pathological environment, they can be subjected to the influence of the surrounding tissue; therefore, within the scar of the postinfarction heart, they can differentiate into connective tissue cells. The plasticity of embryonic stem cells and the other sources of stem cells have become a matter of considerable significance in the study of organ regeneration. However, because of ethical considerations, these cells may be restricted to experimental in vitro studies.

**Bone marrow cells.** BMCs are capable of proliferation, self-renewal, production of a large number of differentiated progeny, and tissue regeneration. Recent studies have shown that several organs, including bone marrow, possess stem cells with greater plasticity than previously envisioned. BMCs display a significant degree of heterogeneity and include bone marrow-residing hematopoietic stem cells and mesenchymal stem cells, which are also known as stromal stem cells, as well as progenitor cells for different tissues. The potential ability to induce both cardiomyogenesis and angiogenesis can be ascribed to both of these main cell subsets.

**Myoblasts.** An interesting type of committed progenitor cells are the tissue reservoir cells known as satellite cells or skeletal myoblasts. They are located in the basal lamina of the adult skeletal muscle. In both preclinical studies and in safety clinical trials, they appear to repopulate the irreversibly damaged postinfarction area. However, because the transplanted skeletal myoblasts remain committed to their lineage, they may retain an action potential duration different from that of adjacent myocardium. This electrical inhomogeneity and the lack of ability of these cells to develop “gap junctions” and to propagate the electrical stimulus may induce new reentrant circuits and predispose to ventricular arrhythmia.

**Delivery systems**

The appropriate route of cell administration to the damaged organ is an essential prerequisite for the success of organ repair. High cell concentrations within the area of interest and prevention of homing of transplanted cells into other organs are desirable. Therefore, targeted and regional administration and transplantation of cells should be preferred. Below, several special routes of administration are described.

**Intramyocardial injection.** Orlic et al. studied the repair of infarcted myocardium in mice using highly enriched stem/progenitor cells from male mouse bone marrow. The left coronary artery of female mice was ligated and 5 hours later Lin-c-kit+ BMCs, obtained from male mice expressing enhanced green fluorescent protein (EGFP), were injected into the healthy myocardium adjacent to the site of the infarct. After 9 days the damaged hearts were examined for regenerating myocardium. A band of new myocardium was observed in 12 surviving mice. The developing myocytes were positive for EGFP, Y chromosome, and several myocyte-specific proteins including cardiac myosin and cytoplasmic and nuclear markers of cell differentiation (GATA-4, MEF2, and Csx/Nkx2.5). The cells were also positive for connexin-43, a gap junction intercalated disc component, indicating the onset of intercellular communication. Myocyte proliferation was demonstrated by incorporation of 5-bromo-deoxyuridine (that identifies cell nuclei in the S phase) into the DNA of dividing cells and by the presence of the cell cycle-associated protein Ki67 in their nuclei. Neovascularization was also observed in regenerating myocardium. Endothelial and smooth muscle cells in developing capillaries and small arterioles were EGFP-positive. No myocardial regeneration was observed far from the infarct area and in damaged hearts transplanted with Lin-c-kit- BMCs, which lack bone marrow-regenerating activity. Several hemodynamic parameters exploring contractile function of the repaired left ventricle were significantly improved.

Stamm et al. used bone marrow stem cell transplantation as a new means to restore tissue viability after myocardial infarction. In 6 post-AMI patients undergoing coronary bypass grafting, these authors injected up to 1.5 × 10⁶ autologous AC133+ BMCs into the infarct border zone. AC133 reflects the transformation of circulating endothelial progenitor cells into more mature endothelial-like cells. Three and 9 months after surgery, all patients were alive and well, global left ventricular function had improved in 4 patients, and infarct tissue perfusion had improved strikingly in 5 (Table I). The authors concluded that implantation of AC133+ stem cells is safe and might induce angiogenesis, thus improving perfusion and func-

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From Stamm et al., modified.
tion of the infarcted myocardium. However, their conclusion has to be taken with caution. In fact, their patients underwent both stem cell implantation and surgical revascularization of non-infarcted, ischemic myocardium. Therefore, the relative contribution of improved regional perfusion and myocardial regeneration cannot be assessed in this clinical model.

Menasche et al.\textsuperscript{34,35} implanted cultured autologous skeletal myoblasts into fibrotic myocardium at the time of coronary bypass surgery. These cells, which do not raise immunologic, ethical, oncogetic, or donor availability issues, also appear to improve ventricular function (Fig. 2). However, in experimental models employing a similar approach, the presence of connexin-43, a gap junction intercalated disk component, was not demonstrated between grafted myoblasts and host myocytes. Thus, the mechanism of the reported improvement in systolic function is unclear. Other factors, such as paracrine effects, passive girdling and a decrease in wall stress, have to be hypothesized.

However, the encouraging preliminary results obtained by these authors opened the way to the first clinical trial in coronary disease patients with low ejection fraction, akinetic and non-viable postinfarction scars, elected to coronary bypass surgery in remote, viable, and ischemic areas. Large-scale cell expansion allows a yield of $>10^9$ myoblasts from a single human muscular biopsy. Cultured autologous myoblasts are directly administered by multiple injections within and around the infarct area during open-chest surgery. Preliminary postoperative observations show an improvement in ejection fraction, reappearance of systolic thickening of the grafted scars, and new-onset metabolic activity within the previously non-viable area. Thus, this new procedure might become a useful adjunct to current treatments of severe ischemic HF. However, in this early series, a high incidence of both non-sustained and sustained ventricular tachycardia was encountered, which mandated the use of implantable cardiac defibrillators in all “transplanted” patients\textsuperscript{36}.

Transendocardial injection. Perin et al.\textsuperscript{37} evaluated the hypothesis that transendocardial injections of autologous mononuclear BMCs in patients with end-stage ischemic heart disease could safely promote neovascularization and improve perfusion and myocardial contractility\textsuperscript{38,39}. Twenty-one patients were enrolled in this study of whom 14 received the cells and 7 served as controls. Baseline assessment included full clinical and laboratory evaluations, exercise test, Doppler echocardiogram, single-photon computed perfusion tomography, and 24-hour Holter monitoring. Clinical, demographic and exercise test variables were similar in the two groups. Mononuclear BMCs were harvested, isolated, washed, and resuspended in saline solution for injection by a Webster catheter (15 injections of 0.2 ml). Electromechanical mapping by the NOGA apparatus was used to identify viable myocardium amenable to treatment\textsuperscript{40}. Both treated patients and controls underwent 2-month non-invasive follow-up, whilst only treated patients were assessed invasively after 4 months. In the treated group the total area of reversible underperfusion was significantly reduced and global left ventricular function was significantly improved. At 4 months, ejection fraction had improved from a baseline of 20 to 29% and end-systolic volume was reduced. Accordingly, electromechanical mapping revealed a significant improvement of regional wall motion in the injected segments.

Intracoronary infusion. In 10 patients with AMI treated with primary angioplasty Strauer et al.\textsuperscript{31} administered autologous BMCs via a balloon catheter advanced in the infarct-related artery. The cells were injected 7 days after the acute event\textsuperscript{42} and, to maximize extraction by the distal vascular bed, injections were performed during balloon inflation that prevented antegrade flow and rapid washout. Another 10 patients with AMI, and similar clinical characteristics, were treated by primary angioplasty alone and served as controls. In the treated group, the infarct area was significantly smaller on the perfusion scan performed after 3 months (Fig. 3). Regional wall motion was also significantly improved along with stroke volume, left ventricular end-systolic volume, and ejection fraction.

Assmus et al.\textsuperscript{43} randomly allocated 20 patients with reperfused AMI to receive intracoronary infusion of either bone marrow-derived (n = 9) or circulating blood-derived progenitor cells (n = 11) into the infarct-related artery 4 days after AMI. After 4 months, “transplanted” patients showed a reduction in left ventricular systolic volume with a significant increase in ejection fraction.
the normal healing process would allow. However, all the reported studies involved small series, they were not randomized and had no real control groups. Therefore, the results of larger prospective randomized trials need to be available, before these techniques become established. Furthermore, their effects on long-term mortality need to be addressed.

Systemic mobilization. On the basis of previous investigations, Orlic et al.\textsuperscript{44} concluded that two critical determinants are required for the transdifferentiation of primitive BMCs: tissue damage and high concentrations of pluripotent cells\textsuperscript{45}. They hypothesized that BMCs, mobilized by stem cell factor and granulocyte-colony stimulating factor, would home in the infarct region, replicate, differentiate, and ultimately promote myocardial repair. Indeed, in the presence of experimental myocardial necrosis in the mice, cytokine-mediated translocation of BMCs resulted in a significant degree of tissue regeneration within less than 1 month. In the treated animals, cytokine-induced cardiac repair decreased mortality by 68\%, infarct size by 40\%, cavity dilation by 26\%, and diastolic stress by 70\%. Ejection fraction progressively increased (Fig. 4) and hemodynamics significantly improved as a consequence of the formation of $15 \times 10^6$ new myocytes supplied by neoangiogenetic arterioles and capillaries connected with collaterals originating from the unaffected myocardium. Therefore, mobilization of primitive BMCs by cytokines may potentially represent a non-invasive therapeutic tool for regenerating irreversibly injured ischemic myocardium.

More support to this hypothesis was provided by Shintani et al.\textsuperscript{46} who showed that circulating CD34\+ monocytes significantly increase in patients with AMI. Plasma concentrations peaked 7 days after the acute event suggesting that a further increase in these cells, stimulated by the administration of specific cytokines, and improved regional wall motion. In the control group, left ventricular ejection fraction increased only slightly and end-systolic volume remained unchanged. In the treated patients, coronary flow reserve of the infarct area was also significantly increased and quantitative F-18-fluorodeoxyglucose positron emission tomography revealed a significant increase in the amount of viable tissue in the infarct zone (Table II). The improvement in all tested variables was similar regardless of whether the patients had received blood-derived or bone marrow-derived progenitor cells. Apparently, the cells did not elicit an inflammatory response, nor they induced malignant ventricular arrhythmias.

Catheter-based cell transfer to the human heart has unique advantages. It is a safe procedure that can be performed during routine cardiac catheterization, and adds very little risk and burden to a standard procedure. It allows the administration of large amounts of BMCs to the infarct region, providing the heart with a greater availability of stem cells in a short period of time than

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EDV = end-diastolic volume; ESV = end-systolic volume; FDG-PET = F-18-fluorodeoxyglucose positron emission tomography; LVEF = left ventricular ejection fraction. From Assmus et al.\textsuperscript{41}, modified.

Figure 3. Perfusion scan in a patient with recent acute anterior myocardial infarction at baseline (A, B) and 3 months after bone marrow cell transplantation (C, D). The anterior wall exhibits a significant improvement in myocardial perfusion. From Strauer et al.\textsuperscript{41}, modified.

Figure 4. Ejection fraction during three different follow-up times in control mice (SO), non-treated infarcted mice (AMI) and cytokine-treated infarcted mice (AMI+C). * p < 0.05 vs AMI. From Orlic et al.\textsuperscript{44}, modified.
may enhance cardiac repair and induce angiogenesis, therefore preventing eccentric remodeling and residual ischemia.

To identify a source of stem cells capable of repairing damaged cardiac tissue, Jackson et al. transplanted highly enriched hematopoietic stem cells, the so-called side population cells, into lethally irradiated mice subsequently rendered ischemic by coronary artery occlusion for 60 min followed by reperfusion. The engrafted side population cells or their progeny migrated into the ischemic cardiac muscle and blood vessels, differentiated to cardiomyocytes and endothelial cells, and contributed to the formation of functional tissue. Side population cells were purified from Rosa26 transgenic mice, which expresses lacZ gene widely. Donor-derived cardiomyocytes were found primarily in the peri-infarct region with a prevalence of around 0.02% and were identified by expression of lacZ and alpha-actinin. Donor-derived endothelial cells were identified by expression of lacZ and Flt-1, an endothelial marker known to be absent on side population cells. Endothelial engraftment had a prevalence of around 3.3%, and occurred primarily in small vessels adjacent to the infarct area. These results demonstrate the cardiomyogenic and angiogenetic potential of hematopoietic stem cells and may provide yet another alternative for cell transplant in the failing myocardium.

Kocher et al. showed that bone marrow from adult humans contains endothelial precursors endowed with phenotypic and functional characteristics typical of embryonic hemangioblasts. These cells can be collected after cytokine administration and can be used to directly induce neoangiogenesis in the infarct area and proliferation of preexisting vessels after experimental myocardial infarction. Neoangiogenesis resulted in decreased apoptosis of hypertrophied myocytes in the peri-infarct region, long-term salvage and survival of viable myocardium, reduction in collagen deposition, and sustained improvement in cardiac function. Therefore, the use of cytokine-mobilized autologous human bone-marrow-derived angioblasts has the potential to reduce left ventricular remodeling.

Conclusions

Myocardial cell replacement therapy for the treatment of patients with postinfarction HF appears feasible, and initial observations justify further research. In particular, randomized, prospective, placebo-controlled clinical trials are warranted to objectively assess the results of these interventions. Before that, moreover, many issues are still to be solved. For instance, the optimal model of stem cell delivery is currently unknown, though catheter-based or direct surgical approaches seem the most suitable for human use. Furthermore, the best timing for implant, the number and the type of cells and the characteristics of patients who are going to benefit most from the treatment are still to be established. In fact, stem cell differentiation into connective tissue, new vessels or myocytes may be determined by the local environment encountered by these cells at the time of implant. Also, whether stem cell therapy would be most beneficial early after infarction where significant inflammation coexists, later in the remodeling phase, or perhaps at the end stage of ischemic cardiomyopathy is not known. Current data appear to indicate that, for clinical applications, BMCs are, probably, the best option when transplanted early after infarction.

Riassunto

Il trapianto di cellule staminali è stato proposto come strategia sperimentale per il trattamento di patologie cardiache quali l’infarto miocardico acuto (IMA) e l’insufficienza cardiaca, in considerazione dell’altissimo impatto sociale e della mancanza a tutt’oggi di una terapia risolutiva. Infatti, sia la terapia della fase acuta, medica e/o interventistica, che della fase cronica sono ancora, per diversi motivi, non pienamente efficaci. Lo scompenso cardiaco, naturale evoluzione della cardiopatia ischemica, rappresenta uno dei maggiori problemi di salute pubblica nei paesi industrializzati in quanto patologia cronica, progressiva, associata ad elevati costi per l’elevata mortalità e morbilità.

Recenti tentativi di riparare un IMA, sperimentalmente indotto in animali da laboratorio o spontaneo nel modello umano, hanno fornito risultati incoraggianti, seppur limitati. L’utilizzo delle cellule staminali, sia in fase acuta che cronica, potrebbe risultare, insieme al trapianto cardiaco, l’unica opzione terapeutica apparentemente efficace per sostituire il miocardio infarctuato ed evitare il conseguente rimodellamento sfavorevole. Ad oggi, sia sull’uomo che sulla cavia, sono state testate diverse sorgenti cellulari per ottenere la rigenerazione di miociti contrattili: cellule staminali midollari, embrionali o fetales, oltre a mioblasti scheletrici. L’efficacia è valutata in base alla sopravvivenza e alla maturazione delle cellule trapiantate, all’accoppiamento elettromeccanico con il miocardio preesistente ed agli effetti sulla funzione globale a breve e lungo termine. L’approccio terapeutico può essere mirato a contrastare la fase acuta (IMA) o cronica (scompenso cardiaco) della cardiopatia ischemica con diverse vie di somministrazione.

La terapia cellulare della cardiopatia ischemica presenta, al momento, ancora molti aspetti da approfondire. Per prima cosa è da stabilire in quale stadio di malattia i pazienti possano trarre il maggiore beneficio dalla terapia con cellule staminali. Si può, quindi, ipotizzare che durante la fase acuta (IMA) si verifichino un maggiore rilascio di fattori chemiotattici dal microambiente della regione infartuata e sia già presente una quota maggiore di cellule midollari mobilitizzate con un maggior numero di miociti in fase di mitosi spontanea.
Una somministrazione troppo precoce in corso di IMA potrebbe, però, contribuire allo sviluppo dei processi infiammatori già presenti durante i primi giorni. Per contro, intervenire nel momento in cui sia già presente scompenso cardiaco conclamato rende qualsiasi approccio terapeutico meno efficace poiché il processo di rimodellamento si è già avviato.

La mobilizzazione di precursori cellulari circolanti mediante fattori di crescita rappresenta sicuramente una via semplice e sicura in considerazione del comune utilizzo di questa tecnica in donatori sani di midollo osseo. Gli approcci invasivi, seppur più complessi, permettono di posizionare le cellule là dove servono. Un aspetto ancora non chiarito riguarda il tipo cellulare da utilizzare. Le cellule staminali midollari possono agire sia sulla rigenerazione diretta del miocardio che stimolando la neoangiogenesi. I mioblasti scheletrici, seppur facilmente estraibili, hanno evidenziato un minore spettro d’azione, legato alla sola rigenerazione diretta del miocardio, ed un’importante limitazione legata al loro effetto proaritmico. Tutte le fonti cellulari per la rigenerazione di nuovi miociti presentano, comunque, incognite comuni: sopravvivenza a lungo termine, differenziazione e capacità di raggiungere un fenotipo maturo, capacità di integrarsi funzionalmente con il mio-cardio ospite e di contribuire alla funzione contrattile, risposta adeguata a stimoli fisiologici e patologici, oncogenicità e proaritmia.

In conclusione, i dati preliminari ottenuti in pazienti con IMA suggeriscono che le esperienze raccolte nel modello animale potranno essere trasferite entro breve nell’ambito clinico.

References