Stem cell transplantation for ischemic myocardium

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Despite recent advances in interventional cardiology (brachytherapy and drug-eluting stents) and in cardiac surgery (less invasive "off-pump" techniques), there is still a percentage of patients who cannot benefit from any of these treatments. These patients may ultimately develop end-stage chronic heart failure or untreatable and invalidating angina. Since replacement techniques such as heart transplantation or ventricular assist devices are still burdened by a shortage of donors and health care resources, cell transplantation is gaining a growing interest among research groups.

Cell transplantation is based on two major assumptions: 1) heart failure develops when a critical number of cardiomyocytes has been irreversibly lost, and 2) function can thus be improved by repopulating these areas of "dead" myocardium with a new pool of contractile cells. One of the most compelling observations that validates this concept of functional replacement therapy derives from studies of Langerhans islet transplantation in diabetic patients. These studies show that after a median follow-up of 10.2 months, 11 of 12 patients intraportally injected with allogeneic islets and receiving appropriate immunosuppressive therapy were able to achieve insulin independence[1].

However, we may assume that spontaneous multiplication of adult cardiomyocytes, if any[2,3], is far too low to compensate the loss of infarct-injured cells and that conversion of in-scar fibroblasts into contractile cells would require genetic manipulations of questionable clinical relevance. Therefore, the most realistic approach consists of exogenously supplying a new pool of contractile cells and engrafting them into the postinfarction scars. The requirement for a discrete area to target cell injections has resulted in most of the preclinical studies using models of segmental ischemic cardiomyopathies. On the other hand, preliminary data suggest that the benefits of cell transplantation may also extend to the setting of globally dilated, idiopathic[4], or drug-induced[5] cardiomyopathies.

The prerequisite for implanted cells to improve cardiac function is that they feature contractile properties. Although some positive data have been reported with fibroblasts[6], smooth muscle cells[7], and endothelial cells[8], these results remain inferior to those yielded by contractile cells where this contractility is naturally present such as fetal cardiomyocytes derived from embryonic stem cells, obtained from the inner cell mass of the blastocyst[9]. However, their use is still debated and troubled by major problems. First, there are ethical problems. Second, the need for immunosuppressive therapy after cell transplantation, due to their heterologous origin. Third, their terminal differentiation and potential malignity are, at present, unknown.

Another source of contractile cells is provided by autologous skeletal myoblasts. The early results with such cells seem to be encouraging[10]. This therapeutic approach offers the possibility of autologous transplantation from the patient's own skeletal muscle without immunosuppressive therapy. However, the implantation of skeletal myoblasts has been shown to provoke ventricular tachyarrhythmias[11], thus justifying a word of caution regarding their use.

Transplantation of bone marrow cells is giving rise to a growing interest because these cells share with myoblasts the possibility of being used as autografts and they could have the additional advantage of a

transdifferentiation potential allowing them to convert into myocardial or endothelial cells or both. Stamm et al.[12] showed a striking gain in left ventricular ejection fraction and improved diastolic left ventricular dimensions in 4 patients at transthoracic echocardiography, and an improved perfusion in the previously non-perfused or hypoperfused infarct zone in 5 patients at single-photon emission computed tomography.

Transplantation of total, unfractionated bone marrow is clinically appealing because of its simplicity. This technique entails aspiration of bone marrow from the iliac crest and, after removal of red blood cells, immediate reinjection of the aspirate into the postinfarction scar. This procedure has already been used in patients but so far only some preliminary data have been reported in abstract form[13]. Although few details are available, the results seem to demonstrate the feasibility of the technique rather than its efficacy.

The second option is to select well-defined populations of hematopoietic progenitors with the assumption that their plasticity would allow them to transdifferentiate in response to the environmental cues present in the target organ, and more specifically, to convert into cardiac or endothelial cells or both after engraftment in the myocardium. This theory has been primarily advocated by Orlic et al.[14,15] who reported "regeneration" of infarcted mouse myocardium by Lin-c*kit*^{POS} cells injected intramyocardially or endogenously translocated by cytokines.

A third option is to use bone marrow mesenchymal cells, which are easy to collect and expand. In both rat[16] and swine[17] infarction models, these cells have been shown to differentiate into cardiac and blood vessel cells, which correlated with an improved regional perfusion and wall motion, greater scar thickness, and an augmented global heart function. However, a method for stromal cells to acquire a myogenic phenotype is their pretreatment, during their growth in culture, by 5-azacytidine (a demethylation agent that can cause an out-of-control upregulation of a wide variety of genes and as such raises clinically relevant safety concerns), even if this pre-treatment is not always considered as an obligatory prerequisite for their commitment to cardiomyocytes[18,19]. Yet another method is the intercellular interaction between adult human mesenchymal stem cells and adult human cardiomyocytes that would induce stem cells to acquire the phenotypical characteristics of cardiomyocytes[20].

So far, cell injections have usually been accomplished under direct control through multiple epicardial punctures. To reduce the invasiveness of the procedure, percutaneous approaches are undergoing a largely industry-driven extensive development. Much emphasis is put on endoventricular injections that benefit from improvements in catheter design and navigation systems. Surprisingly, the growing number of patients undergoing these procedures (often as live cases during well-attended meetings) sharply contrasts with the paucity of robust animal data showing (as has been the case for epicardial injections) that this "blind" approach is not only technically feasible but also functionally efficacious. A recent experimental study[21] has reported a higher intramyocardial retention of microspheres after endoventricular injections compared with epicardial injections but whether these results may be extrapolated to the use of cells remains uncertain. In the setting of these percutaneous techniques, the transvenous approach using a specific coronary sinus catheter is particularly attractive because of its greater simplicity compared with the endoventricular route.

Regardless of the route of delivery, cell death remains a major limitation of cell transplantation as up to 90% of cells may die shortly after injection and it is uncertain whether multiplication of those that have survived can make up for this high attrition rate[22]. Several factors contribute to cell death including physical strain during injections, inflammation, apoptosis, and the hypoxic environment inherent in postinfarction scars. Mangi et al.[23] genetically engineered rat mesenchymal stem cells using *ex vivo* retroviral transduction to overexpress the prosurvival gene Akt1 (encoding the Akt protein). Transplantation of cells overexpressing Akt into the ischemic rat myocardium inhibited the process of cardiac remodeling by reducing intramyocardial inflammation, collagen deposition and cardiac myocyte hypertrophy and completely normalized the systolic and diastolic cardiac functions.

Nevertheless, the importance of the hypoxic environment is illustrated by the finding that the survival of cardiomyocytes grafted into highly vascularized granulation tissue is 2-fold higher than that observed after grafting into acutely necrotic myocardium[24]. Therefore, we believe that cell-transplanted segments should be concomitantly revascularized as much as possible (either percutaneously or surgically) in an attempt to optimize the benefits of cell transplantation by providing them with a vascular environment.

In our preliminary clinical experience we treated 5 patients from February to May 2002 with bone marrow cell transplantation during revascularization procedures using "off-pump" surgery; we did not use cardiopulmonary bypass in order to prevent the myocardial metabolic damage and the modifications of the vascular environment distinctive of the "on-pump" technique and cardioplegia. Among these patients, we recorded one pure "responder" (with an improved regional perfusion and wall motion in the area of cell injections alone) and one "non-responder". In the remaining 3 patients we recorded an improved perfusion and wall motion in the area of cell injection, but it was not possible to determine whether these regional and global improvements were due to the associated revascularization or to cell transplantation alone. At present, all 5 patients are doing well (mean follow-up 20.8 ± 0.8 months) and no late complications have been recorded so far (unpublished data).

In conclusion, bone marrow cell transplantation for ischemic myocardium is a safe and feasible procedure and preliminary data about its efficacy have been increasingly reported.

Cell death remains the major limitation of this technique; probably, the timing of injection affects cell survival and there may be an optimal time window for cell transplantation. Too-early postinfarction injections may fail because of a high rate of cell death due to the infarct-induced inflammatory reaction and late injections may be equally ineffective because of the inability of cell grafts to reverse the remodeling process once it has been completed[25].

Therefore, the right timing of cell injection in relationship with myocardial infarction as well as the appropriate delivery system seem to be the crucial issues at present. Furthermore, the survival rate of the transplanted cells needs to be elucidated by means of labeling techniques or by measuring the neo-innervation of the myocardium[26]. In conclusion, we do believe that stem cell transplantation may become an appealing treatment for patients whose coronary artery disease does not render them eligible for conventional revascularization techniques.

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