Adult stem cells therapy for the heart

Uso terapêutico de células-tronco em Cardiologia

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ABSTRACT

Acute myocardial infarction causes irreversible loss of cardiomyocytes and endothelial cells compromising the cardiac function of patients. For some patients, current therapeutic approaches are not sufficient to prevent myocardial remodeling, which aggravates the disease. The potential of many types of adult bone marrow-derived progenitor cells in replacing damaged myocardium with functional cells has been investigated in several clinical and experimental studies. It has been demonstrated that these cells have an overall beneficial effect on heart function although the mechanism or multiple mechanisms involved have not been elucidated yet. Here, it will be reviewed some aspects of this fast-growing and controversial field of adult autologous progenitor/stem cells for cardiac repair.

Keywords: Stem cells; Heart; Myocardial infarction

ACUTE MYOCARDIAL INFARCTION AND CELL DEATH

Cardiovascular disease remains one of the leading causes of death in the world³. The occlusion of one or more coronary arteries, which are responsible for heart muscle blood supply, causes acute myocardial infarction (AMI). As a consequence of AMI, different areas of the heart are affected in distinct ways and degrees of severity depending on various factors, particularly the intensity and duration of the ischemic event³. In early time-points (minutes to hours) following the insult, intense and indiscriminate cell loss (cardiomyocytes, endothelial and nervous cells) leads to thinning of heart walls with systemic and local release of inflammatory mediators²-³.

The amount of mediators released is proportional to the size of injury and these elements participate directly in the recruitment of inflammatory cells into the affected region. The inflammatory response is activated aiming repair. However, this initial response amplifies the lesion process by causing additional necrosis. In parallel, cardiomyocytes submitted to a lower degree of ischemia undergo cell death by apoptosis (reviewed in³). Although apoptosis leads to cardiac muscle loss, it may somehow help to control the secondary inflammatory response to AMI. Once acute inflammation is abated, long-term repair and a healing process take place. Healing occurs mainly by scar formation. Large numbers of fibroblasts appear at the site of injury, leading to collagen deposition and formation of extensive areas of fibrosis. Neovessel formation is also observed. However, although circulation might be reestablished, fibrotic areas do not contribute to global myocardial performance due to loss of contractile units. The consequent process of combined modifications in size, shape and function of the heart following injury is named ventricular remodeling³.

Once remodeling is established, and sometimes in spite of adequate clinical management, a decline in left ventricular function ensues. To compensate...
the significant loss of functional cardiomyocytes, a transcriptional program observed during heart development is reactivated\(^4\). However, in adult hearts the activation transcription factor gene expression usually linked to heart development (e.g. GATA-4, MEF2 and Nkx2.5) generates cardiomyocyte hypertrophy instead of cell duplication (reviewed in\(^5\)). Cardiac hypertrophy refers to increases in size and mass of individual cardiac myocytes without increments in cell number. Hypertrophy occurs as a reparative and adaptive process. However, it frequently evolves into changes in ventricular geometry and contributes to remodeling and development of heart failure\(^6\).

Coronary artery disease is not usually restricted to the area directly affected by the infarction. In addition to epicardial vessels, myocardial microcirculation is also often compromised limiting blood flow and; therefore, oxygen delivery. Thus, the degree of ischemia associated with the infarct-related artery is not the only variable involved in the determination of chronic myocardial dysfunction. Even when epicardial vessel disease is amenable to mechanic revascularization, blood supply may not be adequately restored. The decrease in cardiac performance activates neurohormonal compensatory mechanisms. Activation of the sympathetic system (through an increase in the concentration of catecholamines) is aimed to maintain cardiovascular function by increasing heart rate, myocardial contractility and peripheral vascular resistance. Activated renin-angiotensin-aldosterone system also leads to vasoconstriction (angiotensin II) and an increase in blood volume, due to salt and water retention (angiotensin II, aldosterone) (reviewed in\(^7\)). If sustained, neurohormonal activation promotes additional cardiac remodeling, with intense myocardial and vascular fibrosis and cardiomyocyte hypertrophy.

Lately, the general paradigm that the number of myocytes in the heart is determined at birth and that it cannot increase throughout the organism life has been challenged. Convincing evidence indicates that heart has an endogenous, yet limited self-renewal potential. In the past few years, different populations of undifferentiated progenitor cells have been identified in the post-natal heart, in favor of a cardiac progenitor cell (CPC) compartment\(^8\). CPCs are implicated in the physiological replacement of myocytes, endothelial and smooth muscle cells in the heart. Furthermore, myocardial regeneration has been demonstrated by identification of male recipient cell engraftment into transplanted female donor hearts\(^9\). The chimerism in transplanted hearts suggests that male progenitor cells migrate to the female heart and, then, differentiate into myocytes and endothelium. This observation suggests that naturally occurring circulating adult progenitor cells can be mobilized to the heart where they participate in the process of cardiac recovery. The discovery of CPCs and the existence of additional natural mechanisms of myocardium repair generated enthusiastic perspectives in Cardiology. Many researchers are currently attempting to clarify the mechanisms of cardiac homeostasis, the potential therapeutic use of resident CPCs and why endogenous heart repair mechanisms are not sufficient to regenerate hearts of patients following myocardial infarction.

Despite advances in the pharmacological treatment, no therapeutic approach has shown efficacy in replacing myocardial scar with functioning contractile cardiomyocytes. Recent progresses in stem cell biology and regenerative medicine have triggered the interest in using stem cells to repair injured myocardium. Left ventricular dysfunction resulting from ischemic cardiomyopathy has distinct components, including irreversibly compromised myocardial regions and areas of viable, dysfunctional myocardium that can have their function restored. Multiplication of healthy, functional cardiomyocytes, neovessel formation, control of apoptosis and fibrosis, and modulation of inflammatory response would be potentially beneficial and are natural goals of cell therapy in Cardiology (Figure 1).

CELL THERAPY IN MYOCARDIAL INFARCTION

In 2001, two independent groups showed that intramyocardial injections of bone-marrow-derived stem cells could improve cardiac function and cell survival, after induced AMI\(^11\). Since then, several types of progenitor/stem cells have been used in experimental studies to promote post-infarction myocardial repair (Table 1). These studies have employed embryonic
stem cells, fetal and adult cardiomyocytes, skeletal myoblasts, bone marrow cells (BMC), peripheral blood cells, multipotent adult progenitor cell (bone marrow non-hematopoietic adult stem cells, named MAPCs) and mesenchymal stem cells. Surprisingly, the injection of any of those distinct cells types into and around the site of injury led to a relatively homogeneous final outcome, with an overall improvement in the contractile function of the left ventricle (Table 1). Thus, it is not surprising that an important pending question in this field of research has been the determination of the best source of stem cells for cardiac therapy. The majority of experimental and clinical studies focused on the use of whole bone marrow (BM) or bone marrow-derived stem cells, because of their autologous origin and their alleged transdifferentiation potential into cardiac and endothelial cells. At least three different well-characterized types of stem cell populations are found in the bone marrow, and can account for the observed transdifferentiation: hematopoietic stem cells (CD34+, CD133+), endothelial progenitors (CD133+/VEGFR2+) and mesenchymal stem cells (MSC) (CD34- and CD133-).

MSC are a rare population of self-renewing, multipotent cells found in the bone marrow, and more recently also identified in several other tissues, including fat and umbilical cord (blood and vessel wall). There is an increasing interest in the use of mesenchymal stem cell therapy for myocardial infarct, since it was first observed that they could differentiate into cardiomyocytes in vitro after 5-azacytidine treatment(13).

Besides, their multidifferentiation potential, high expansion capability and low immunogenicity make MSCs good candidates for stem cell cardiac therapy. Many experimental studies have shown that, when injected into myocardium after MI, MSCs engraft into the infarcted region, express muscle-specific markers and improve left ventricular contractility. However, Dai et al. have shown that six months after MSCs injection into the heart, they did not evolve completely to cardiomyocytes in spite of expressing muscle-specific markers(13). These data indicate that MSCs do not assume a mature cardiomyocyte phenotype in the infarcted heart and suggest that mechanisms, other than differentiation, contribute to the observed beneficial effects on heart function. It has been proposed that MSCs improve heart function via paracrine effects. In fact, that seems to be a reasonable explanation since it has been shown that MSCs secrete a large number of arteriogenic cytokines and growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (BFGF), placental growth factor and monocyte chemoattractant protein (MCP-1)(14).

### Clinical Trials Using Adult Progenitor Stem Cells for Acute Myocardial Infarction

Recently, many clinical trials for the treatment of AMI tested the therapeutic potential of the injection of adult progenitor/stem cells into the injured heart of patients following myocardial infarction (Table 2). Clinical trials were quickly initiated based on the exciting observations

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**Table 1.** Rodent models of acute myocardial infarction using bone marrow-derived progenitor cells with positive outcome

<table>
<thead>
<tr>
<th>Study</th>
<th>Cell type</th>
<th>Transdifferentiation</th>
<th>Fusion</th>
<th>Paracrine factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jackson et al. (11)</td>
<td>BMCS SP</td>
<td>Yes</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>Orlie et al. (12)</td>
<td>BMCS Lin-, c-kit+</td>
<td>Yes</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>Toma et al. (21)</td>
<td>Human BM-MSCS</td>
<td>Yes</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Agbolut et al. (22)</td>
<td>BMCS</td>
<td>Yes</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Kudo et al. (23)</td>
<td>BMCS/Lin-</td>
<td>Yes</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>Davani et al. (24)</td>
<td>BM-MSCS</td>
<td>-</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>Saito et al. (25)</td>
<td>BM MSCS</td>
<td>Yes</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>Hisao et al. (26)</td>
<td>BM-MNCS</td>
<td>No</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>Nygren et al. (27)</td>
<td>BMCS CD34+, CD133+, Lin-, c-kit+, Sca-1+</td>
<td>No</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Murry et al. (28)</td>
<td>BMCS c-kit+ Lin-</td>
<td>No</td>
<td>ND</td>
<td>Yes</td>
</tr>
<tr>
<td>Baslam et al. (29)</td>
<td>BMCS c-kit+ Lin- and BMCS c-kit+ Lin- Sca-1+ Thy.1+/Lin-</td>
<td>No</td>
<td>ND</td>
<td>No</td>
</tr>
<tr>
<td>Kajstura et al. (30)</td>
<td>BMCS c-kit+</td>
<td>Yes</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>Noiseux et al. (31)</td>
<td>BM-MSCS overexpressing Akt</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Tang et al. (32)</td>
<td>BM-MSCS</td>
<td>Yes</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Martens et al. (33)</td>
<td>Human BM-MSCS-STRO-1+</td>
<td>Yes</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>Uemura et al. (34)</td>
<td>BMSCs</td>
<td>Yes</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Mirotou et al. (35)</td>
<td>BM-MSC overexpressing Akt</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Yang et al. (36)</td>
<td>BM-MSC overexpressing VEGF</td>
<td>Yes</td>
<td>-</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not determined; BM: bone marrow; MSCS: mesenchymal stem cells; BM-MNCS: bone marrow mononuclear cells; LIN: lineage
of neoangiogenesis and myogenesis, associated with improved contractile function reported after transplantation of BM stem cells (BMC) and progenitor cells in animals, models of myocardial infarction\(^ {11-12}\). Although the mechanisms behind the improved cardiac function were not determined, clinical needs for more effective forms of treatment and the promise of stem cell therapy formed the initial basis of what is now thought to be a rushed translation into clinical studies.

The results of the freshly published randomized placebo-controlled clinical trials (Table 2) provide a realistic perspective on cardiac stem cell therapy and accentuate the need to unveil the biological basis of this promising approach. The improvement in the left ventricular function observed in patients infused with BMC-derived cells ranges from no significant difference to 6\% in terms of ejection fraction\(^ {15-21}\), figures which, at first glance, do not seem very impressive. However, it should be considered that those clinical trials were conducted in a scenario of full standard pharmacological therapy. Despite the diversity of parameters (type of cell, number of cells, way of cell infusion, timing, etc) and the inconsistency among the clinical and experimental studies, the collective analysis of the papers indicates that transplantation of progenitor cells from BM or circulating blood progenitor cells is a safe and feasible approach that may lead to improvements in the heart function (Table 2).

In phase I clinical trials, autologous BMC or blood peripheral progenitor cells were injected due the practicability of using patients’ own cells. Even after at least a dozen clinical studies, it is hard to achieve a consensus of which type of autologous population tested would be the most effective for treating AMI (Table 2). In the TOPCARE -AMI pilot study\(^ {22}\), the treatment with mononuclear BMCs and ex vivo expanded circulating progenitor cells led to similar outcomes when transplanted immediately after myocardial infarction. However, in another clinical trial by the same group (TOPCARE-CHD) with patients who had a myocardial infarct at least three months before cell transplant, the performance of circulating progenitor cells was significantly inferior to that seen with BMCs in ameliorating left ventricular function\(^ {15}\).

Table 2. Clinical studies using autologous blood- and bone marrow-derived cells for acute myocardial infarction

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Cell type/treatment</th>
<th>Dose (number of cells)</th>
<th>Time of cell administration (post-AMI)</th>
<th>Type of study</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAGIC Cell Kang et al.(^ {48})</td>
<td>2007</td>
<td>GCSF-mobilized PBSC</td>
<td>7 x 10(^ 6) CD34+</td>
<td>Variable</td>
<td>Randomized</td>
<td>+</td>
</tr>
<tr>
<td>BOOST Wollert et al.(^ {18}) Meyer et al.(^ {17})</td>
<td>2004</td>
<td>BMMNC</td>
<td>2.5 x 10(^ 4)</td>
<td>4-8 days</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Janssens et al. (^ {21})</td>
<td>2006</td>
<td>BMMNC</td>
<td>2 x 10(^ 6)</td>
<td>2 days</td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td>TOPCARE Assmus et al.(^ {15-18})</td>
<td>2006</td>
<td>PBSC</td>
<td>2 x 10(^ 6)</td>
<td>3 months and 6 months</td>
<td>+</td>
<td>+/BMMNC</td>
</tr>
<tr>
<td>REPAIR-AMI Schachinger et al.(^ {18})</td>
<td>2006</td>
<td>BMMNC</td>
<td>2 x 10(^ 6)</td>
<td>3-7 days</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ASTAMI Lunde et al.(^ {21})</td>
<td>2006</td>
<td>BMMNC</td>
<td>7 x 10(^ 7)</td>
<td>4-8 days</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Bartunek et al.(^ {48})</td>
<td>2005</td>
<td>CD133 + BMC</td>
<td>1.3 x 10(^ 7)</td>
<td>13 days</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Katritsis et al. (^ {84})</td>
<td>2005</td>
<td>MSC and EPC</td>
<td>2-4 x 10(^ 6) (66% MSC and 28% EPC)</td>
<td>Variable</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Erbs et al.(^ {50})</td>
<td>2005</td>
<td>GCSF-mobilized PBSC</td>
<td>7 x 10(^ 6) CD34+</td>
<td>&gt; 30 days</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Boyle et al.(^ {82})</td>
<td>2005</td>
<td>GCSF-mobilized CD34+ cells</td>
<td>7 x 10(^ 6) CD34+</td>
<td>Variable</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Galinanes et al.(^ {93})</td>
<td>2004</td>
<td>Whole BM</td>
<td>10(^ 6)</td>
<td>3 months and 6 months</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Chen et al.(^ {54})</td>
<td>2004</td>
<td>MSC</td>
<td>5 x 10(^ 10)</td>
<td>18 days</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fernandez-Aviles et al.(^ {51})</td>
<td>2004</td>
<td>BMMNC</td>
<td>8 x 10(^ 7)</td>
<td>14 days</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ozbaran et al.(^ {54})</td>
<td>2004</td>
<td>GCSF-mobilized PBSC</td>
<td>13 x 10(^ 6)</td>
<td>Variable</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fuchs et al.(^ {57})</td>
<td>2003</td>
<td>Unfractionated BMC</td>
<td>3 x 10(^ 6)</td>
<td>&gt; 1 month</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Stamm et al.(^ {54})</td>
<td>2003</td>
<td>CD133 +</td>
<td>3 x 10(^ 6)</td>
<td>10 days-3 months</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tse et al.(^ {54})</td>
<td>2003</td>
<td>BMMNC</td>
<td>Variable</td>
<td>Not determined</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Strauer et al.(^ {34})</td>
<td>2002</td>
<td>BMMNC</td>
<td>3 x 10(^ 7)</td>
<td>7 days</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

N: neutral; BMC: bone marrow cells; BMMNC: bone marrow mononuclear cells; EPC: endothelial progenitor cells; PBSC: peripheral blood stem cells; MSC: mesenchymal stem cells

\(^ {15}\) to 6\% in terms of ejection fraction
\(^ {21}\) figures which, at first glance, do not seem very impressive. However, it should be considered that those clinical trials were conducted in a scenario of full standard pharmacological therapy. Despite the diversity of parameters (type of cell, number of cells, way of cell infusion, timing, etc) and the inconsistency among the clinical and experimental studies, the collective analysis of the papers indicates that transplantation of progenitor cells from BM or circulating blood progenitor cells is a safe and feasible approach that may lead to improvements in the heart function (Table 2).

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Looking at the design of the randomized clinical trials, we observe a lack of objective criteria during the process of defining the amount of cells required to reach optimal therapeutic outcome (Table 2). The evaluation of results of different trials shows no clear correlation between the quantity of infused cells and improvement in left ventricular function, as it would be expected in a classical dose-response effect.

One of the longest follow-up clinical trial reported to date, the BOOST (BM transfer to enhance ST-elevation infarct regeneration) study, showed that the significant improvement in the left ventricular ejection fraction observed in the group infused with BMCs, at six months, was no longer significant after 18 months\(^{(17)}\). In that study, the Control Group, at later time points, achieved the same level of functional recovery observed in treated individuals. These findings challenge the hypothesis that expressive formation of new myocardial tissue, following cell transplant takes place after cell therapy. More likely, the results suggest that the main effect seen was due to a process of speeding up the functional recovery that already occurs with standard pharmacotherapy.

Skeletal myoblasts were first thought to be an advantageous cell type for heart infusion, because of their contractile phenotype, possibility of autologous transplantation, ability of *ex vivo* expansion and resistance to ischemia. Clinical and experimental studies have shown that the infusion of skeletal myoblasts leads to improvement in symptoms and left ventricular ejection fraction. However, the same studies have disclosed a high frequency of ventricular arrhythmia and sudden death\(^{(23-24)}\), questioning the safety of using myoblasts for heart therapy.

### PARACRINE FACTORS

Although clinical trials have been initially designed to produce myocardial regeneration via transdifferentiation, recent experimental results suggest that the improvement observed after BM-derived cell infusion is more likely to be due to the release of paracrine factors by transplanted cells. In fact, studies show a variety of putative mechanisms by which BM-derived cells improve heart function (Table 1).

Using more rigorous criteria for ruling out transdifferentiation than those employed in earlier reports, two independent groups showed that BMCs genetically labeled did not transdifferentiate after being transplanted into injured mouse hearts. In actuality, they assumed mature hematopoietic phenotypes\(^{(25-26)}\), contesting the idea of transdifferentiation of BMC into cardiomyocytes. In addition, fusion of BMCs with cardiomyocytes seems to be a very rare phenomenon\(^{(27)}\) and cannot be accounted for the improvement in the heart function observed in some studies.

The observation that a wide range of progenitor cell types improve ventricular function after AMI in experimental and clinical settings indicates that benefit may originate from other mechanisms, than *bona fide* myocardial regeneration. Lately, the focus in the field has changed from myocardial regeneration to remodeling attenuation therapy triggered, accelerated and/or maintained by paracrine effects of transplanted cells.

It has been hypothesized that paracrine factors would have a protective effect on the surviving myocardium and/or contribute to neovessel formation. Paracrine factors already participate in the physiological tissue repair of the injured heart, by either providing homing signals for naturally occurring circulating progenitor cells or through activation of resident progenitor cells\(^{(28)}\). Normally, after an acute myocardial infarction, plasma concentrations of vascular growth factors such as VEGF, BFGF, and the stem cell homing factor SDF-1 increase gradually in response to hypoxia, reaching a peak few weeks after the infarction. SDF-1 has been found to be essential for stem cell mobilization/homing after arterial injury. In a recent experimental study, it has been demonstrated that SDF-1 gene transfer increased homing of BMCS to infarcted myocardium, but not to normally perfused hearts\(^{(29)}\). Interestingly, the TOPCARE-AMI study showed that the capacity of stem cells in improving heart function was related, particularly, to their migratory activity rather than the cell number transplanted\(^{(30)}\). The abovementioned study also suggested that positive cells for the SDF-1 receptor CXCR4 participate in heart regeneration.

Experimental studies have shown that hematopoietic stem cells can also be mobilized to infarcted myocardium by SCF, the ligand for c-kit, with additional improvement in cardiac function. Fazel et al.\(^{(31)}\) showed that bone marrow-derived c-kit cells are involved in a naturally occurring process of heart function restoration in mice, by a mechanism that is independent of transdifferentiation into either cardiac muscle or endothelial cells. The authors observed that in c-kit mutant mouse model, an animal that carries a deletion in the transmembrane domain of it in one allele and a missense mutation in the kinase domain of c-kit in the other allele, there is impairment in HSC mobilization capability, even in the presence of normal numbers of stem cells. Of interest, a more significant cardiomyopathy following AMI occurs in mutant animals when compared to normal, wild-type mice. In addition, the failing hearts in c-kit defective mice could be rescued by BM reconstitution with wild-type BMC. Heart function improvement observed was not associated with long term engraftment of BMCs, but...
rather with release of angiogenic cytokines and increase of neovascularization in the injured myocardium.

In humans, after a myocardial infarction, an increased number of CD34-positive cells (hematopoietic and endothelial progenitors) are mobilized into the circulation\(^{[32]}\). This physiological mobilization of CD34-positive cells does not result in effective myocardial repair. Since granulocyte colony-stimulating factor (GCSF) mobilizes multipotential progenitor cells of BM into peripheral blood, there has been some interest in administrating GCSF after myocardial infarct. It is conceivable that GCSF would enhance the number of progenitor cells in the peripheral circulation, increasing the probability of their homing to the region of injured myocardium, thus maximizing the improvement in left ventricular remodeling and function. Ripa et al. studied the effects of GCSF administered subcutaneously in the post-infarction period in a randomized, double-blind, placebo-controlled trial\(^{[33]}\). They found that BM stem cell mobilization with subcutaneous GCSF did not improve ventricular function after AMI.

Myocardial infarction is followed by an inflammatory response that leads to additional myocardial damage, healing and scar formation. The therapeutic effect of BMCs and other progenitor cells may be related to the acceleration of cardiac scar formation by affecting inflammatory pathways, influencing preferentially healing than regeneration. In this case, a stronger immune response would be advantageous for myocardial repair after MI. The short-term therapeutic effect of BM observed in the BOOST trial\(^{[17]}\) might be based on an improvement in an already existent inflammatory response, followed by enhanced release of cytokines and nonspecific angiogenic factors. The synergistic combination of host and transplanted cell-mediated release of paracrine factors would provide an optimal pattern of cytokines and growth factors, accelerating cardiac regeneration as observed in the aforementioned recent clinical trials.

There is few data concerning the role of host immune response in the modulation of the therapeutic effect of adult progenitor cells in post-infarcted myocardium. Tolar et al. showed that whereas labeled MAPCs persist longer in the post-infarct myocardium of immunosuppressed mice (mice lacking T, B, and NK cells) when compared to immune competent animals, the left ventricular function was improved only in mice with the immune system intact\(^{[34]}\). These results indicate that the use of immunosuppression in allogeneic or xenogeneic cell-based therapies for AMI should be viewed with caution, as it may hamper the therapeutic effects of stem cells.

Successful regeneration of infarcted heart with cell-based therapy remains a promise. It must be kept in mind, however, that most of the approaches used in clinical trials were not able to generate sufficient mature myocardium to promote significant functional improvement. The most important lesson from experimental and clinical studies of cell therapy for the heart is that we understand very little about the mechanistic basis for heart regeneration. Consequently, there is still a great deal of basic research to be done. To determine the paracrine factors released by adult progenitor cells and whether they are responsible for the therapeutic effects in the cardiac recovery, observed in clinical and experimental studies, it is necessary to identify and measure changes in specific factors following cell therapy in experimental models of myocardial infarction. There is also the need to clarify the reason for the disparity between the exceptional results obtained in the majority of the experimental studies and the timid results from large randomized clinical trials.

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