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Myocardial Infarction: Cell Therapy for Cardiac Regeneration

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Abstract

Mortality rate in patients that suffer heart failure is approximately 50 per cent in a 5-year follow up, exceeding the mortality detected in patients with cancer. Angiotensin Converting Enzyme (ACE) inhibitors and beta-blockers are effective to treat Myocardial Infarction (MI), but there is no effective therapy to reverse the disease. In the last two decades, cell therapy has emerged as an important treatment to be considered for patients with MI. In the present Review, I will summarize the diversity of cell therapies that have been used in pre-clinical and clinical studies, discussing the pros and cons of each therapy.

Keywords: Myocardial infarction; Cardiovascular disease; Cardiomyocytes; Cell therapy

Introduction

Cardiovascular disease is the main cause of mortality worldwide accounting for 17.3 million deaths in 2008 and an estimated number of 23.3 million in 2030, according to the World Health Organization [1]. The most common cardiovascular disease is heart failure due to Myocardial Infarction (MI). The reduction or complete deprivation of oxygen and nutrients experienced after an ischemic infarct leads to a massive death of cardiomyocytes in the affected area. This region is rapidly repopulated with migrating myofibroblasts, responsible for the deposition of extracellular matrix proteins that will form the scar area. Different strategies have been tested in pre-clinical studies, in order to delay or interfere with the adverse ventricular remodeling and recover the loss of working myocardium. Delivery of growth factors and cytokines [2-5], *in vivo* reprogramming of myofibroblasts and dividing non-cardiac cells into cardiomyocytes [6,7], or cell therapy are among the treatments that have shown marginal results.

Two decades ago, the publication of studies demonstrating that C2C12 myoblasts can engraft in the murine heart [8] and mouse fetal cardiomyocytes can engraft and form intercalated disks with host myocardium [9], was a milestone that triggered the first studies using cells as a treatment for MI. Since that time, thousands of papers have been published in the field (more than five thousand references retrieved from PubMed introducing the terms "Cell therapy for Myocardial Infarction") and dozens of clinical trials.

In this review I will give an update of the most relevant work performed using cell treatment after MI, discussing challenges, advantages and disadvantages of each approach.

Cell Therapy Strategies

Bone marrow cells

In the late 90's and early 2000's, Bone Marrow Cells (BMCs) emerged as the master key for regenerative medicine. They were reported to be capable of differentiating or transdifferentiating *in vivo* into hepatocytes [10], brain cells [11] and cardiomyocytes [12,13] among other cell lineages. Differentiated cardiomyocytes even showed positive staining for the gap junction protein connexin 43 (Cx43) [12], as a proof of functional connection between the new formed muscle cells in the scar area of infarcted mice. However, attempts to reproduce these results by other groups were unsuccessful [14-17]. In the particular case of the heart, 9-10 days and 28-30 days post-implantation, donor BMCs were positive for the pan-hematopoietic marker CD45 [15,17] and the granulocyte marker Gr1[15]. Nevertheless, there was no evidence of cardiac, smooth muscle cell or endothelial cell transdifferentiation [15], in contrast with previously published studies [12,13]. Moreover, different groups reported that the process described *in vivo* as BMC transdifferentiation was actually the product of cell fusion between BMCs and host organ cells [17-21], shattering the proposed plasticity of BMCs.

The study by Orlic et al. [12] describing an occupancy of 68% of the infarcted area by transplanted BMCs and enhanced left ventricular performance of animals in which they were implanted, prompted many groups to initiate the first clinical trials [22,23]. TOPCARE-AMI was the first randomized pilot clinical trial that involved 59 patients divided into two groups receiving unfractionated BMCs or circulating progenitor cells [22,24]. In a 5-year follow-up report [24] the authors detected no differences between both groups of patients but a clear improvement in the left ventricular ejection fraction (LVEF) at 4 months that was maintained until the 5 years with respect to the baseline, in a cohort of 31 patients. Unfortunately, the design of this study lacked a control group to compare the relevance of the obtained results [22,24]. In the ASTAMI clinical trial, a 3-year follow-up [25] showed no differences in LVEF between control and BMCs infused groups. Interestingly, they observed an increase in LVEF at 3 months in both control and cell-treated groups, highlighting the importance of controls in experimental designs. From more than a dozen clinical trials in progress, only 4 of them have provided long term results (>2 years).

ASTAMI [25] and BOOST [26] showed no changes in BMCs treated patients with respect to the control group at 3 and 5 years respectively. REPAIR-AMI [27], although did not find LVEF differences, it did find

a significant reduction in infarct size and increased wall thickening of infarcted regions 2 years after BMCs implantation. The results from the fourth clinical trial, TOPCARE-AMI, with a 5-year follow-up have been described above [24]. More detailed reviews about clinical trials, including the cell delivery numbers and routes of administration can be found in Behfar et al. [28] and Pavo et al .[29].

Clinical trials with BMCs have shown a very modest effect as shown in a recent meta-analysis performed in 16 clinical trials [30] with only a 2.55% increase in LVEF in patients treated with BMCs with respect to the corresponding controls. Strikingly, another meta-analysis study [31] considering 49 clinical trials, reported discrepancies in the vast majority of the trials (all except 5). In this study, the authors showed a correlation between the number of discrepancies per trial and the effect on ejection fraction size, concluding that in free-discrepancies clinical trials, BMCs had zero effect on ejection fraction [31]. In a recent meta-analysis, performed in 12 randomized studies with intracoronary cell administration of autologous cells, and using original Individual Patient Data (IPD), authors concluded that no benefit was observed in cell-treated patients [32].

In summary, BMCs' therapy has been demonstrated to be safe and feasible, but showed reduced [30] or no effect [31, 32] in patients' LVEF.

Myoblasts

In 1961 Mauro described a population of cells surrounding differentiated myofibers that he named "satellite cells" [33]. These cells lie beneath the basal lamina of myotubes and when cultured *in vitro* they differentiate to myoblasts [34]. Myoblasts have been studied exhaustively as cell therapy for MI, in part due to their ability to survive, proliferate and finally differentiate to skeletal muscle in the harsh environment present in the infarcted heart [35]. Undifferentiated myoblasts express N-cadherin and Cx43 [36], proteins that are involved in cell adhesion and gap junction formation in intercalated disks. Nevertheless, both proteins are lost when myoblasts differentiate into skeletal myotubes [36], and it has been demonstrated that engrafted myotubes are electromechanically isolated from host myocardium [37]. Several groups have reported in pre-clinical studies the beneficial effects of implanted myoblasts in different MI models [38, 39]. Considering that myoblasts do not transdifferentiate into cardiomyocytes in infarcted animals [40], it has been suggested that their mechanism of action is through secretion of factors that interfere with the adverse ventricular remodeling [41]. Myoblasts' translation to the clinic was performed for the first time in 2001 [42] showing the feasibility of myoblast implantation in one patient with MI. Since 2001, several clinical trials were started. In a four-year follow-up study, delivery of myoblasts in patients undergoing concurrent Coronary Artery Bypass Grafting (CABG) or Left Ventricular Assist Device (LVAD) implantation was compared [43]. Patients with myoblast implantation and CABG presented an improvement in LVEF and tissue viability. However, it is difficult to interpret these results since a control group is missing in the study. In other clinical trials in which control groups were included, patients with myoblast implantation did not have any effect in LVEF [44,45]. In both studies, the presence of ventricular arrhythmias in the treated group was the main concern exposed [44,45].

Adipose tissue-derived cells

Adipose tissue is composed of mature adipocytes and a Stromal Vascular Fraction (SVF). The SVF contains vascular cells and a population of Mesenchymal Stem Cells (MSCs). MSCs have potential to differentiate spontaneously into cardiomyocytes *in vitro*, with rare events described [46], and endothelial cells *in intro* and *in vivo* [47-49]. Adipose-tissue Derived Cells (ADCs) represent an attractive strategy for cell therapy due to the large amount of cells that can be isolated from each patient with a minimally invasive technique as liposuction [50]. Pre-clinical studies in mouse [51] and pig [49] using cell sheet [51] or direct injection in the coronary artery [49], have shown increased LVEF in animals treated with adipocytes [51] or Adipose-tissue Derived Stem Cells (ADSCs) [49]. When compared with BMCs, both BMCs and ADSCs groups presented a significant increase in LVEF, but only ADSCs presented a significant increase in wall thickness with respect to control group [49]. Of interest is the fact that neither ADSCs nor BMCs treated pigs showed cardiomyocyte differentiation of donor cells [49]. The proposed mechanisms of action of adipose tissue-derived cells are angiogenesis [49] and secretion of paracrine factors like adiponectin, who may regulate extracellular matrix production by myofibroblasts [51].

On the clinical side, there is only one study reported with a very modest effect of adipose tissue-derived cells: the PRECISE trial [50]. This randomized, placebo-controlled and double-blinded study enrolled 27 patients: 21 treated with Adipose-derived Regenerative Cells (ADRCs, the SVF of adipose-tissue), and 6 controls. No differences were detected in LVEF at different time points within the group and neither between groups. Authors reported a significant increase in left ventricular total mass in ADRC-treated patients at 6 months with respect to the baseline [50].

Cardiac progenitor cells

A Cardiac/Cardiovascular Progenitor Cell (CPC) is a cell that, after losing its stemness properties, is committed to differentiate at least into the three main lineages of the cardiovascular system: cardiomyocytes, endothelial cells and smooth muscle cells. Many different laboratories have claimed the isolation of CPCs from fetal [52] and adult hearts [52,54], or after Embryonic Stem Cells (ESCs) *in vitro* differentiation [55, 56]. CPC isolation based on cell surface markers and their existence in the adult heart has been a continuous matter of debate. The most common markers used to isolate adult and ESC-derived CPCs are: 1) c-kit (also known as stem cell factor receptor; SCFR or CD117), 2) the stem cell antigen-1 (Sca1) and 3) the fetal liver kinase-1 (Flk1, known as well as vascular endothelial receptor 2 or KDR in humans). The three surface markers are present in hematopoietic stem cells [57-59]. Researchers that question about their true CPC identity consider that these CPCs found in adult hearts might be just circulating bone marrow-derived cells homing in the heart.

Recently, using lineage tracing studies, a group has shown that c-kit may not be appropriate to identify CPCs since they minimally contribute to cardiomyocytes in the heart [60]. Although a Sca1 human orthologue has not been identified yet, scientists have isolated cells from adult human hearts using an antibody against mouse Sca1 [52]. Sca1+ cells were able to differentiate *in vitro* into cardiomyocytes, although using non-conventional methods for cardiac differentiation: demethylating agent 5-azacytidine (5-aza) [52]. Notably, 5-aza induces cardiac differentiation in the mouse embryonic carcinoma cell line P19 [61], murine BMCs [62] and human mesenchymal stem cells [63]. Therefore, several cell types from diverse origins are able to differentiate into cardiomyocytes in the presence of 5-aza.

Lineage tracing studies have provided more information about Sca1+ derived progeny [64]. In this study, Sca1-derived cardiomyocytes were first detected, at low numbers, 2 months after birth, but Sca1+ cells were unable to mobilize to the infarcted area after MI [64]. The virtual absence of Sca1-derived cardiomyocytes until postnatal stages reinforces the idea that at least fetal cardiomyocytes are not derived from Sca1+ cells. Flk1 lineage tracing studies have shown that, in the heart, they contribute

mainly to the endocardium, although some cardiomyocytes were also stained [65]. However, Flk1 may not be an optimal marker to isolate ESC-derived CPCs since it has been recently reported that it is an early marker of hepatocyte progenitor cells [66].

In 2003 Anversa's laboratory described the presence of c-kit+ cells in adult rat hearts, which had the potential to differentiate *in vitro* to cardiomyocytes, smooth muscle cells and endothelial cells [53]. According to the results presented, these c-kit+ cells, after *in vitro* expansion, were able to engraft and differentiate into cardiomyocytes in a rat model of MI, improving the LVEF of infarcted animals [53]. c-kit+ cells extracted from adult hearts were translated to clinic under the name of SCIPIO [67]. This clinical trial, with 16 patients treated with cells and 7 control patients, reported very encouraging results. In patients treated with cells, the LVEF increased 8.2 and 12.3 units at 4 months and 1 year respectively [67]. The reader should be aware that an expression of concern has been raised by Lancet editors regarding this work [68].

Anversa's group results were first challenged after a new study reported that c-kit+ cells contribute to myocyte formation in neonatal but not in adult MI [69].

In 2007, Eduardo Marban's group described the isolation of cells from endomyocardial biopsies that form cardiospheres after expansion on poly-D-lysine coated plates [54]. These Cardiosphere-Derived Cells (CDCs) are a heterogeneous population of cells that express among other markers, c-kit and CD105 and do not contract spontaneously in culture [54]. Extensive literature has been generated using the so called cardiospheres, showing improvement of heart function in different animal models of MI [54,70]. CDCs (CD105+ cells) are under study in the clinical trial CADUCEUS [71]. In this study 31 patients were randomized, 23 of them treated with autologous CDCs (17 after removing some technical failures) and 8 controls. No differences were found at 1 year in LVEF and the only significant effect found was decreased scar size in CDC group versus control group at 1 year [71].

Although both clinical trials have proven that cells extracted from adult hearts, either c-kit+ [67] or CD105+ cells [71], are a safe therapy for patients with MI, many more pre-clinical studies are necessary in order to identify cell surface markers for the unambiguous isolation of authentic CPCs capable of differentiating into cardiomyocytes with conventional differentiation methods.

ESC-derived Cardiomyocytes

Another strategy being pursued by scientists, still in pre-clinical studies, is the replacement of dead myocardium with fully differentiated cardiac cells. Initial studies were conducted using fetal and adult cardiomyocytes [72-74] showing long-term engraftment of fetal and neonatal [72-74] but not adult [73] cardiomyocytes in the infarcted area. Remarkably, rat neonatal cardiomyocytes were rod-shaped 8 weeks after implantation, with presence of N-cadherin and Cx43 in the cell-cell contact regions, resembling adult cardiomyocytes [73].

The isolation in 1998 of the first human ESCs (hESCs) lines from human blastocysts [75] and the posterior development of the initial protocols for human cardiac differentiation [76] opened up the door for exploring the potential of hESC-derived cardiomyocytes (hESC-CMs) for MI. hESC-CMs were able to couple electromechanically with neonatal rat cardiomyocytes *in vitro* and pace the heart in a swine model of complete atrioventricular block [77]. Later, hESC-CMs tested in rat models of MI, presented long-term engraftment and improvement of heart function with respect to control rats [78,79]. In these studies hESC-CMs were injected at 4 days [78] or 7-10 days [79] after coronary ligation in the presence [78] or absence [79] of a cocktail of prosurvival factors. Interestingly, while one of the studies reported that the majority of the grafts were in the infarct border [79], the presence of grafts in the other one was mainly inside the scar area [78]. The successful results obtained in rats were reproduced in bigger animals with slower heart rates. Using engineered-hESC-CMs expressing the calcium sensor GCaMP3, researchers were able to demonstrate that hESC-CMs couple electromechanically with host myocardium in a guinea pig [80] and non-human primate [81] models of MI. Although the results obtained in the guinea pig model were encouraging and researchers observed arrhythmia-suppressive effects of hESC-CMs grafts [80], the presence of arrhythmic processes was raised as one of the major concerns found in non-human primates treated with hESC-CMs [81]. Despite the considerable engraftment of hESC-CMs, covering 40% of the scar volume, and the electromechanical graft-host coupling observed in non-human primates [81], the arrhythmic events probably due to the inability of hESC-CMs to acquire a mature phenotype *in vivo*, may delay the translation of hESC-CMs to clinical studies.

The ethical concerns raised after the isolation of hESCs from human blastocysts [82] have been overcome with the advent of the induced pluripotent stem cell (iPSC) technology [83]. Currently, iPSCs can be generated using transgene-free, genome integration-free technologies like the RNA-based Sendai virus vector [84]. After hESC/iPSC differentiation, a few cells may remain undifferentiated; being a potential source for teratoma formation. Different methods attempted to eliminate undifferentiated cells, and recently, *Lee et al.* [85] developed a clinical-grade strategy using small molecules against survivin. These molecules selectively eliminate undifferentiated cells, without interfering with the differentiation process [85]. Human iPSC-cardiomyocytes can be obtained with high purity (90%) in the laboratory [86] and could be readily used applying patient-specific therapy, once the arrhythmic events reported in non-human primates [81] have been solved.

Conclusions

Since the first studies, performed almost two decades ago, that proved the feasibility of cell therapy [35,72] as a new approach to be considered in MI treatment, a wide diversity of cell types have been tested in pre-clinical studies. BMCs, ADCs and myoblasts were attractive sources because of the possibility of extracting large amounts of cells for autologous therapy. BMCs and ADCs showed beneficial effects in cardiac function through the induction of angiogenesis and secretion of paracrine factors in the infarcted heart [49,51,87]. Myoblasts proliferate and differentiate in MI pre-clinical models [35] interfering with adverse ventricular remodeling [41], but are not able to couple electromechanically with host cardiomyocytes [37]. To date, there is no evidence of BMCs, ADCs or myoblasts-mediated induction of host cardiac proliferation and none of them differentiate or transdifferentiate into cardiomyocytes *in vivo* [15,40,49].

CPCs' attractiveness resides in the fact that they can give rise not only to cardiomyocytes but also smooth muscle cells and endothelial cells [88], with the potential to form blood vessels, which are required for cardiac graft survival. Many laboratories have reported the isolation of fetal, adult or hESC-derived CPCs [52,53,56] based on cell surface markers that are not specific for the cardiovascular lineage [57,60,66]. Close collaboration with developmental biology laboratories is required to better help us to identify cell surface markers that are unique to CPCs.

hESC-CMs are able to repopulate large areas of infarcted myocardium and couple electromechanically with host cardiomyocytes [81] in a non-human primate model of MI, positioning them as an excellent cell source for future clinical trials. Improvement in cell engraftment, specific isolation

of ventricular cardiomyocytes and search for strategies to enhance hESC-CM maturation *in vivo*, in order to alleviate ventricular arrhythmic processes would help this therapy to jump from bench to bedside.

Translation of cell therapies into clinical studies has not been as rewarding as pre-clinical studies predicted. Different factors may be involved in this fact, from techniques used to isolate cells before implantation, density gradients [24] and Ficoll [26] to differences in number of cells implanted ranging from half million [68] to more than two billion [26]. Regarding the number of cells, the POSEIDON trial reported an inverse dose response effect in LVEF [89]. It should be considered as well that controlled conditions of "patients" in pre-clinical studies cannot be achieved in clinical studies.

In summary, cellular therapies for MI have been proven to be safe in clinical trials [24-27,50,71], but it is a matter of debate if they confer any beneficial effect for patients [30-32]. These results suggest that the race to find the ideal cell type for MI is still more open than ever before.

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Footnotes

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I apologize to scientists whose wonderful work is not represented in this article. This review article was conceived to give a general overview of this continuously expanding field.

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