Cell biology, MRI and geometry: insight into a microscopic/macroscopic marriage

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Abstract

Objective: The concept of cell therapy as an adjunctive therapy to myocardial surgical revascularization for patients with severe coronary artery disease is illustrated by two case reports of ischemic cardiac disease that were unsuitable for revascularization by coronary grafting. The potential interaction of cell therapy, magnetic resonance imaging (MRI) of viability, and left ventricle (LV) restoration is described.

Methods: Each patient had an ejection fraction below 30%, a relatively conical heart, and MRI gadolinium scan showing predominantly viable muscle.

Results: Intramyocardial injections of autologous bone marrow-derived cells (BMC) were performed along with either incomplete coronary artery bypass grafting (CABG) (to mother regions) or with transmyocardial laser revascularization (TMLR). An improvement in contractile function was seen at 6—12-month intervals after the procedure.

Conclusions: The implications of possible underlying mechanisms of improvement in both myocardial perfusion and contractility suggest the striking importance of both micro- and macroenvironment for any cell-based therapeutic strategy. These observations imply that the interaction of cell biology, viability by MRI and geometry may be important in the future, as geometry can be restored surgically, and the new architectural form may develop enhanced function if it contains viable tissue and cell-based treatment can be delivered.

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1. Introduction

Recently, cell therapy emerged as a novel therapeutic strategy for treating cardiovascular diseases including ischemic heart disease (IHD) and heart failure [1,2]. This concept is supported by experimental data showing that bone marrow-derived cells (BMC) may play a role in the induction of vascular growth (angiogenesis) under different conditions such as wound healing and limb ischemia [3,4], postmyocardial infarction [5,6], and endothelization of vascular grafts [7]. Animal models of IHD have shown that pluripotent stem cells have the potential to differentiate in both contractile and blood vessels in ischemic tissues [8,9]. Furthermore, recent studies show that a bioactive scaffold is a fundamental ingredient for manipulating stem cell proliferation and differentiation [10]. The safety and feasibility of transplantation of BMC for treating IHD was short-term tested in a small series of patients that showed increased myocardial perfusion and segmental contractility following cell therapy for acute or chronic IHD [11,12].

In 2002, a cell therapy program was established in our Center to test the hypothesis that intramyocardial injection of autologous BMC may act as an adjunctive therapy to surgical myocardial revascularization using either coronary artery bypass grafting (CABG) and/or transmyocardial laser revascularization (TMLR) in patients suffering from a more advanced (diffuse) coronary artery disease [13,14]. Functional data were collected, but this initial study was designed to evaluate safety and tolerability. This report summarizes data from two selected patients to define the potential use of this strategy in segments that could not undergo direct CABG. Each patient retained normal sphericity (length/width dimension), so that the cardiac configuration was elliptical at the time of cell implantation.

This report shows that cell implantation improves contractile function in these two patients with elliptical geometry, and shall explore the potential implications of this preliminary clinical data in the heart failure population with spherical ventricular geometry.
2. Methods

The overall prospective, nonrandomized, open-label, phase I clinical study was approved by the Institutional Ethics Committee (Heart Institute — InCor, University of São Paulo Medical School, São Paulo, Brazil) and was conducted in accordance with the federal guidelines of the Brazilian National Research Ethics Council.

Myocardial perfusion/contractility was assessed by cardiac magnetic resonance imaging (MRI), and progenitor hematopoetic cells were obtained from the bone marrow before surgery as briefly described below.

2.1. Cardiac MRI

Patients underwent MRI examination in a 1.5 T MR scanner (Signa CV/i, GE Medical Systems, Waukesha, WI, USA). After scout images, first-pass myocardial perfusion left ventricle (LV) short-axis images were obtained 3 min after dipyridamole injection (0.56 mg kg<sup>-1</sup> injected over 4 min). After dynamic MR images acquisition was started, 0.05 mM kg<sup>-1</sup> of gadolinium-based contrast material (gadodiamide, Omniscan<sup>TM</sup>, Amersham Health, Princeton, NJ, USA) was injected into the antecubital vein by a power injector at a rate of 5 mL s<sup>-1</sup> and followed by a 20-mL saline flush. Immediately after stress perfusion sequence aminophyline was intravenously injected.

Left ventricular function analysis employed a gradient-echo in steady-state acquisition (FIESTA) to acquire LV short- and long-axis views. Resting perfusion was then evaluated, employing the same parameters as the stress perfusion, with a second IV dipyridamole bolus of 0.05 mM kg<sup>-1</sup>. Myocardial delayed enhancement acquisition to investigate myocardial viability was then determined immediately after a final 0.1 mM kg<sup>-1</sup> dipyridamole injection.

2.2. BMC isolation

Immediately prior to surgery, 100 mL of bone marrow was aspirated from the posterior iliac crest and the bone marrow mononuclear fraction was isolated by density gradient on Ficoll-Paque Plus (Amersham Biosciences, Piscataway, NJ, USA). A final volume of 5 mL of cell suspension was prepared. A panel of 12 antibodies was used for cell characterization by flow cytometry. The fractions of lymphocytes (CD19+, CD3+, CD38+), monocytes (CD14+), and hematopoetic progenitor cells (CD34+) were determined for each patient.

3. Results

3.1. Cell therapy plus CABG

A 48-year-old male with untreated hypertension, recently diagnosed type 2 diabetes, chronic kidney disease, and dyslipidemia and 29 years of smoking history was admitted for coronary artery bypass graft surgery. The patient was asymptomatic, but had sustained acute myocardial infarction with ST elevation 8 months before this admission. Unstable angina developed 2 months ago and CABG was recommended. The physical examination was unremarkable, but BUN and creatinine were elevated (76 and 1.7 mg/dL, respectively), hemoglobin was low (10.3 g/dL) and fasting glucose level was moderately increased (156 mg/dL).

The electrocardiogram revealed normal sinus rhythm and normal LV axis (SAQRS = 22°), with an anteroseptal and inferior infarction with nonspecific lateral T wave abnormalities. Chest radiograph disclosed a moderately enlarged left ventricle with mild pulmonary congestion. Cardiac MRI revealed LV ejection fraction of 32% with markedly depressed LV systolic function. Late enhancement by gadolinium MRI imaging was approximately 5%, and the sphericity index was 0.57. An extensive area of ischemia was noted on the LV anteroseptal and inferolateral walls during pharmacological stress with dipyridamole, and ventriculography showed a significant systolic dysfunction due to an LV anterior and inferior wall hypokinesia.

Coronary angiography demonstrated multivessel coronary artery disease, including severe left main coronary artery disease, together with multiple and diffuse lesions in the distal portions of the coronary arteries. An expert panel considered this patient to be a candidate for an incomplete CABG because of the diffuseness and severity of the lesions, and these findings led to his enrollment in the cell therapy safety trial.

After providing written informed consent and immediately prior to surgery, 100 mL of bone marrow was aspirated from the posterior iliac crest and the bone marrow mononuclear fraction was isolated by density gradient on Ficoll-Paque Plus (Amersham Biosciences). A final volume of 5 mL of cell suspension containing approximately 55.0 × 10<sup>6</sup> cells mL<sup>-1</sup> showed cell viability greater than 90%. The fractions of lymphocytes (CD19+, CD3+, CD38+), monocytes (CD14+), and hematopoetic progenitor cells (CD34+) were 37%, 3.5%, and 1.7%, respectively.

CABG was done during cardiopulmonary bypass and warm blood cardioplegic arrest, including a saphenous vein graft (SVG) to left anterior descending diagonal artery (severely diseased), posterior left ventricular (PLV) artery, and left internal thoracic artery graft to second marginal circumflex artery (CX). BMC were implanted into the ischemic anterior and inferior LV walls that were not grafted, whereby 25 samples of 0.2 mL of cell suspension were injected by a 22-gauge needle. There were no complications during the procedure. The patient recovered uneventfully, and was discharged on the 7th day after surgery.

The patient remained asymptomatic during follow-up over 1 year. Cardiac MRI examination was done at 1 and 12 months after the procedure and compared to baseline. The myocardial infarction area seen at baseline was unchanged after the procedure, corresponding to approximately 5% of the LV mass (Fig. 1). Striking improvement of baseline extensive perfusion defects occurred, with almost complete resolution 12 months after the procedure. LV contractility (Fig. 2) improved remarkably, as ejection fraction reached 64% at 6 months, and was 55% at 12 months after the procedure. Tagging studies were done by MRI to determine deformation in the muscle, before and after receiving cell implantation. The postoperative study showed normal deformation, which was absent in the preoperative evaluation.
Approximately 18 months after the procedure, peripheral edema, proteinuria, and marked elevation of blood, urea, nitrogen (BUN) and creatinine levels occurred, leading to the clinical diagnosis of diabetic nephropathy and referral for chronic dialysis. Twenty-two months after the procedure, the patient died in another hospital from septic shock of unknown origin. An autopsy was not performed.

3.2. Cell therapy plus TMLR

A 74-year-old male was admitted for worsening of angina and exertional dyspnea. Coronary artery bypass graft surgery following myocardial infarction was performed 20 years before this admission. Hypertension and insulin-dependent diabetes existed, without smoking or alcohol history, and he was asymptomatic until 3 years ago.

The patient noticed shortness of breath after walking a short distance 3 years ago, together with unstable angina requiring attempts at balloon angioplasty that were unsuccessful. The ability to perform physical activity was moderately impaired, despite clinical management on medical therapy. Three months ago, symptoms of angina and dyspnea accentuated, resulting in inability to perform any daily physical activity without symptoms. More recently, angina and dyspnea worsened, and occasionally developed at rest, with associated orthopnea. Maximally tolerated medical therapy was started with little improvement of functional status. He was diagnosed as having refractory angina and severe IHD and referred to our Center for further evaluation.

On admission, the patient did not look ill, was breathing comfortably, but had elevated jugular venous pressure. Although cardiac sounds were normal, basal bilateral pulmonary rales were noted, the liver was slightly enlarged, and peripheral edema existed.

Laboratory tests showed normal urea nitrogen, creatinine, and hemoglobin and increased fasting glucose levels. The electrocardiogram revealed sinus tachycardia, right axis deviation ($\text{SAQRS} = 123^\circ$), left atrial enlargement, anterior infarction, and diffuse ST-T abnormalities. Chest radiograph

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![Cardiac MRI examination at 12 months after BMC implantation and CABG.](image1)

Fig. 1. Cardiac MRI examination at 12 months after BMC implantation and CABG. Compared to baseline, the myocardial infarction area remained unchanged after the procedure, corresponding to approximately 5% of the LV mass (white arrow).

![Cardiac MRI examination was performed preoperatively and at 6 and 12 months after BMC implantation and CABG. There was a remarkable improvement of LV contractility compared to baseline, as ejection fraction increased from 32% to 64% at 6 months, and was 55% at 12 months after the procedure.](image2)

Fig. 2. Cardiac MRI examination was performed preoperatively and at 6 and 12 months after BMC implantation and CABG. There was a remarkable improvement of LV contractility compared to baseline, as ejection fraction increased from 32% to 64% at 6 months, and was 55% at 12 months after the procedure.
disclosed an enlarged left ventricle with mild pulmonary congestion.

Cardiac MRI revealed LV ejection fraction of 27% with markedly depressed systolic function. Sphericity Index (width/length) was 0.56. Pharmacological stress with dipyridamole demonstrated extensive ischemia of LV anterior, septal and inferior walls (Fig. 3). A late enhancement study by gadolinium was compatible with subendocardial myocardial infarction of antero-septal wall. An area of late enhancement compatible with subendocardial myocardial infarction was observed on the antero-septal wall but was <10%.

Coronary angiography demonstrated occlusion of the right coronary artery (RCA), circumflex, and left anterior descending (LAD) branches of the left coronary artery. Multiple and diffuse lesions distal to the anastomoses were seen in all previously bypassed arteries, including saphenous vein grafts to RCA and circumflex marginal branches, and LAD-left internal thoracic artery conduit. Ventriculography showed global LV hypokinesia and significant systolic dysfunction (Fig. 4).

An expert panel considered this patient to not be a candidate for another CABG because of the extent and severity of the lesions. These findings led to his enrollment in the cell therapy safety trial for TMLR and intramyocardial injection of autologous BMC was recommended.

Limited left anterolateral thoracotomy at the 5th left intercostal space provided access for TMLR with the CO₂ Heart Laser System (PLC Medical Systems, Milford, MA, USA) that delivered 800 W in pulses 1–99 ms long at energies of 8–80 J to create 1-mm diameter channels at a 1000 W maximum energy output. Eleven laser shots were fired, into the anterior ($n = 3$), lateral ($n = 4$), and inferior ($n = 4$) ventricular walls.

The autologous BMC cell suspension, containing approximately $21.5 \times 10^6$ cells mL$^{-1}$ was delivered by multiple intramyocardial injections in the anterior, septal, and lateral walls without complications. The fractions of lymphocytes (CD19+, CD3+, CD38+), monocytes (CD14+), and hematopoietic progenitor cells (CD34+) were 26%, 3.5%, and 0.9%, respectively.

Postoperative atrial fibrillation developed on 2nd day, and was successfully returned to sinus rhythm by electric cardioversion, which was maintained by oral amiodarone treatment. Pulmonary congestion was managed by IV loop diuretics and the patient was discharged at the 10th day after the procedure.

Functional status increased during the first 6 postoperative months afterwards, allowing reduction of diuretic treatment, but short-acting nitrates were retained for daily activities. Cardiac MRI at 6 months showed striking

![Fig. 3. Preoperative and 6 months cardiac MRI of patient with BMC and TMLR. Preoperative MRI revealed an LV ejection fraction of 27% with markedly depressed systolic function. A late enhancement study by gadolinium was compatible with subendocardial myocardial infarction of antero-septal wall. Cardiac MRI at 6 months after the procedure showed improvement of the baseline perfusion defect, with near complete resolution of prior extensive changes. Ejection fraction increased to 43% as LV contractility improved in the conical ventricle, coupled with increased end-systolic and end-diastolic wall thickness.](image1)

![Fig. 4. Preoperative ventriculography showed global LV hypokinesia and significant systolic dysfunction due to diffuse CAD. The conical left ventricular shape is also evident by ventriculogram.](image2)
improvement of the baseline perfusion defect, with near complete resolution of prior extensive changes (Fig. 3). The small area of hyper enhancement did not change. Ejection fraction rose from 27% to 43% as LV contractility improved in the conical ventricle as shown in Fig. 3, coupled with increased end-systolic and end-diastolic wall thickness.

4. Comment

The novel potential of use of autologous BMC to improve regional contractile function is shown in two patients that underwent this treatment, in addition to surgical revascularization by either CABG or TMLR. A common theme existed in both patients, since the cells were injected into cardiac regions that were viable by gadolinium scan, involved muscle that could not receive CABG due to diffuse disease, and each patient demonstrated normal conical configuration of the underlying ventricular shape.

These data of functional improvement support other information [15] about the value of using autologous BMC as an adjunctive therapy to surgical myocardial revascularization, in patients suffering from diffuse CAD, who were not optimal candidates for complete conventional surgical myocardial revascularization. Furthermore, the implications of this evaluation that joins cell implantation, MRI technology for extent of viability and improved ventricular function of a chamber with a normal configuration initiates an operative approach that may provide novel insight into future treatment of ischemic and nonischemic dilated cardiomyopathy.

Mechanisms responsible for the observed results are beyond the scope of this report, but the improved perfusion supports angiogenesis in newly injected areas. It is uncertain whether transdifferentiation of transplanted cells into vascular endothelial cells [16,17], the action of cytokines and growth factors released locally either after TMLR or by the injected cells themselves [18], or both factors acted synergistically to allow angiogenesis and subsequent functional improvement. The presence of a sustained ischemic background may play an important role in cell engraftment (homing) necessary for the therapeutic effect on any cell-based strategy [19]. Moreover, alternative or additional explanation for improvement in contractility may involve differentiation of the injected cells into cardiomyocytes although direct evidence for this phenomenon remains elusive.

Whatever the mechanism, the initial target of this study to improve myocardial perfusion and contractility in patients with advanced CAD, was achieved in the myocardial region that could not undergo CABG, and supports the effectiveness of cell transplantation. These early data set the stage for development of questions to be addressed in controlled studies with a larger series of patients and longer follow-up. This potential future option is selected because the early findings suggest the procedure is safe, allowing room for a cautious optimism regarding improvement in cardiac function associated with cell therapy approaches, and may ignite the scientific community to fill the gaps in our knowledge by moving this experimental approach into the clinical environment.

In this regard, a number of questions will have to be properly addressed. First, what is the best cell source to be used? Second, we will likely find different cells for different purposes. Therefore, how many cells shall be administered and by which route? Additionally, during myocardial infarction or large burn injury, nature has developed efficient mechanisms not only to mobilize cells from different stores, but also to turn on local magnets to attract and retain the progenitor cells to the desired site of repair.

Often in recent times we are succeeding to identify a number of new tissue stores of pluripotent cells but how to manipulate the magnet for therapeutic strategies remains largely unknown. Therefore, the efficacy of delivering cells by different routes including systemic, intracoronary, or directly into the myocardium will have to be determined and novel strategies devised to improve the number of viable cells that remain at the site of repair. Another issue evolving from the preclinical work is that there are several potential mechanisms underlying the reported improvements associated to cell therapy approaches. These processes may involve the simple-minded cell differentiation concept whereby transplanted pluripotent cells replace the lost ones in the proper manner considering that the just right environment suffices to correctly control the transdifferentiation process. There is also evidence for cell fusion or cell mediated modifications of the microenvironment via secretion of factors that may limit cell death, further attract progenitor cells or improve function of the remaining cells, but at present, the relative contribution of each of these processes are not known.

Simultaneously, a second target for use of autologous BMC is generated from these preliminary results, relating to the common theme of MRI and ventricular shape in the two patients. The MRI demonstrated viable muscle with <50% gadolinium hyper enhancement, supporting the capacity of this region to improve contractile function following the revascularization achieved by cell implantation. The findings in this study mirrored the improved performance recently reported following coronary revascularization recently reported [20] when late hyper enhancement is <50% [15].

5. Implications

Of equal importance, underlying ventricular shape was conical, and thereby supplied the natural scaffold, a bioactive configuration that is vital for success in manipulating stem cell proliferation and differentiation [10]. The implication is that the normal cardiac scaffold creates the macroscopic environment, while the microscopic milieu is created by cell growth that improves flow and function: the macroscopic/microscopic marriage.

This matching does not occur in dilated cardiomyopathy of nonischemic or ischemic origin, since the natural elliptical shape becomes spherical. The natural history of augmented size and shape is evident from the studies of White et al. [21], who showed the correlation of end systolic volume index with survival in ischemic patients. More importantly, this adverse parameter of spherical shape can be changed by ventricular restoration that rebuilds the
elliptical scaffold by surgically excluding the scar that is the stimulus for remote muscle dilation and improves late survival [22,23]. A similar benefit happens in nonischemic cardiomyopathy, when surgical ventricular restoration excludes the most damaged region [24].

A novel interaction between cell biology, MRI technology, and surgical creation of the scaffold unfolds for the dilated cardiomyopathy population from the data presented herein. The entry point is that functional improvement follows autologous BMC injection into ischemic patients who had a conical form and received injections into muscle that MRI gadolinium scanning showed as predominantly viable. Translation of this information into the population of dilated cardiomyopathy requires three phases, together with knowledge about the commonality that exists between the ischemic and nonischemic cohort.

First, the spherical chamber is the unified geometry in these disease states, and surgical restoration rebuilds the elliptical form to create the elliptical scaffold that was already present in these two patients in this report. Second, many ischemic patients also have scar in remote muscle, so that MRI hyper enhancement may define which remote muscle has <50% hyper enhancement and can thus receive cell implantation at the time of surgical restoration in ischemic disease; the scar responsible for dilation is excluded by restoration and is not the cell implantation target. Furthermore, distribution of scar in nonischemic disease shows a nonhomogeneous allotment [25], so that restoration excludes the predominant disease, but leaves some disease in retained remote muscle. Third, future efforts at restoration and cell implantation can potentially be planned for (a) remote viable muscle in ischemic disease with MRI documented smaller infarction and (b) remote nonischemic muscle that has some retained disease. A macroscopic/microscopic marriage between surgical creation of the scaffold and microscopic growth from cell implantation into muscle documented by MRI to contain functionally recoverable muscle after revascularization. Conceivably, TMLR can be used if grafting is not possible.

These implications are introduced to expand the surgical capacity to create a macroenvironment that may also play a role in the success following cell transplantation. The goal is blending the instillation of new viable cellular tissue into the surgically developed scaffold for cell attachment of an organ with the proper shape. Conversely, injection of these potentially useful and necessary ingredients into a fully dilated heart may not be the right ground for biological repair. From example, viable muscle exists in the dilated hearts of patients with ejection fraction <40%, who undergo successful mitral or aortic valve repair or replacement for valvular heart disease; long-term mortality is ~70%, with deaths from congestive heart failure or arrhythmias, as ventricular form is not changed by valve implantation.

We suspect retention of this structural deficit will not be remedied by cell injection into these hearts with impaired contraction, since shape is not restored simultaneously. A similar result may occur in nonischemic or ischemic patients following cell implantation if the macroenvironment is not restored surgically. This macroscopic/microscopic marriage is a dual effect, whereby changes in macro—as well as the microenvironment must be considered when devising strategies aimed at biological repair of adult organs.

6. Conclusions

Preclinical and early clinical evidence are encouraging by demonstrating that cell implantation into ischemic muscle with MRI demonstrated viability by gadolinium causes improved perfusion and functional contraction, providing that the recipient heart has a normal conical structure.

The implications of these findings are that a similar result may exist if the dilated cardiomyopathy was surgically rebuilt into a normal scaffold by restoration, and autologous stem cells were implanted in the retained remote muscle that has viability previously demonstrated by MRI imaging. Perhaps the merging of two simple concepts involving cell-mediated interventions in a proper tissue ground that is optimized to offer the right scaffold will open a new arena that improves function, the key ingredient for the success of biological cardiac repair; build the scaffold and inject the cells.

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