Intramyocardial implantation of autologous bone marrow-derived stem cells combined with coronary artery bypass grafting in patients with ischemic cardiomyopathy: a pilot study

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Abstract

Background: Ischemic cardiomyopathy has the distinctiveness of irreversible myocardial damage with scar tissue formation and mainly impaired perfusion of the remaining viable myocardium. We present results of the first series of patients with severe ischemic cardiomyopathy managed in our institution with intramyocardial implantation of autologous bone marrow stem cells at the time of coronary artery bypass grafting. The aim is to evaluate feasibility and safety of the procedure in our institution.

Patients and Methods: Nine patients with severe ischemic cardiomyopathy scheduled for elective coronary artery bypass grafting were managed with concurrent intramyocardial autologous bone marrow stem cells injection in pre-defined viable peri-infarct areas that showed poor perfusion and could not be grafted. Detailed mapping of infarcted and hibernating myocardial segments was performed in all patients with single photon emission computed tomography segmental analysis.

Results: There was no perioperative 30-day mortality. Improvement was evident in left ventricular ejection fraction which was increased significantly from 31.3% preoperatively to 42.4%, 46.6% and 52.5% at 3, 6 and 12 months respectively. Postoperative thallium scintigraphy revealed increased perfusion in myocardial segments corresponding to areas of stem cell injection and a net reduction in the estimated infarct size at 6 and 12 months in 5/8 (62.5%) patients.

Conclusions: Preliminary data from this pilot study show that intramyocardial administration of bone marrow stem cells in patients undergoing coronary bypass grafting for ischemic cardiomyopathy is safe and associated with an improvement in left ventricular function and enhanced reperfusion of non-viable myocardial territories. Hippokratia 2012, 16, 4: 366-370

Key words: cardiac failure, ischemic cardiomyopathy, coronary artery bypass grafting; stem cells, thallium scintigraphy

Introduction

Chronic heart failure is characterized as a modern epidemic. It is estimated that 6-10% of people over the age of 65 suffer from symptomatic heart failure in developed countries. A meta-analysis performed by Gheorghiade and colleagues on 13 multicenter treatment trials, involving over 20,000 patients, revealed that coronary artery disease was the underlying etiology in almost 70% of patients1. Ischemic cardiomyopathy (ICM) has the distinctiveness of irreversible myocardial damage with scar tissue formation and mainly impaired perfusion of the remaining viable myocardium.

Current therapeutic protocols for ischemic heart failure are based on the traditional belief that the heart is unable to generate new cardiomyocytes to replace failing or dying ones, but instead adapts to new stresses by myocyte hypertrophy and cardiac remodelling. Surgical or interventional revascularization represent the mainstay of treatment. Cellular therapy has emerged as a novel potential treatment of severe ischemic heart disease2. Various cell types have been used through epicardial, intracoronary and endocardial route of delivery3. Although the exact underlying mechanisms remain unclear, numerous experimental studies have shown that intramyocardial injection of bone marrow stem cells (BMSC) in ICM is associated with an improvement of left ventricular function and reduction of infarct scar size4. These promising preclinical results led to several clinical trials evaluating possible benefits of stem cell transplantation in humans5.

We present results of the first series of patients with severe ICM managed in our institution with intramyocardial implantation of autologous BMSC at the time of coronary artery bypass grafting (CABG). The aim is to evaluate feasibility and safety of the procedure in our institution.
Patients and Methods

Nine patients with severe ICM scheduled for elective coronary artery bypass grafting were managed with concurrent intramyocardial autologous BMSC injection during the period from January 2009 to September 2011 according to a pre-defined protocol. The study received Institutional Review Board approval and all patients signed written informed consent. Patients were considered eligible for the study if they were between 18 and 79 years of age and were diagnosed with severe coronary artery disease amenable to surgical revascularization according to current guidelines. Echocardiographic criteria included a left ventricular ejection fraction (LVEF) ≤ 40% with a distinct area of dyskinetic or akinetic left ventricular myocardium corresponding to the infarct localization. Detailed mapping of infracted and hibernating myocardial segments was performed in all patients with single photon emission computed tomography (SPECT) segmental analysis. According to the protocol BMSC were implanted in pre-defined viable peri-infarct areas that showed poor perfusion, which could not be grafted due to poor target vessel quality (diffuse atheromatosis, chronic total occlusion, small diameter).

Cell preparation

The day of the operation, after induction of general anesthesia, bone marrow was aspirated from both anterior superior iliac crests after induction of general anesthesia. Handling of the bone marrow after aspiration took place in a good manufacturing practice unit providing a particle-reduced environment of European good manufacturing practice guidelines. Isolation of bone-marrow mononuclear cells (BMMNC) was performed according to a standardized protocol. The enriched cell solution was diluted in patient’s own plasma in a volume of approximately 5 ml. A commercial kit (Stem-Kit™; Immunotech Beckman Coulter, Marseille, France) was used for assessing viability and for enumeration of viable haematopoetic stem cells (CD45+/CD34+), mesenchymal stem cells (CD105+) and endothelial progenitor cells (CD133+). Flow cytometry analysis was performed with the use of a Beckman-Coulter EPICS XL/MCL.

Surgical procedure

All patients were operated on with cardiopulmonary bypass and cardioplegic arrest. The left internal thoracic artery was used for grafting the left anterior descending artery and saphenous vein grafts for the other vessel targets. All coronary arteries with relevant stenoses and sufficient diameter were grafted. After completion of the graft–coronary artery anastomoses, injections of 0.2-0.5 ml of cell suspension using a 22-gauge needle apparatus were made into pre-defined territories of hibernating myocardium at the infarct border zone as guided by detailed preoperative radionucleotide scintigraphic mapping. A swab was used to occlude the injection channel for several seconds to minimize reflux of cell suspension. Immediately after the cell injection, the aortic clamp was removed and the operation was completed as usual. All patients received standard postoperative care in the intensive care unit and cardiothoracic ward.

All patients were closely followed-up for a period of twelve months after discharge. At 3, 6 and 12 months postoperatively echocardiographic evaluation to assess

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Grafted vessels</th>
<th>Volume aspirated (ml)</th>
<th>BMMNC (x 10⁶)</th>
<th>LVEF (%) pre-op</th>
<th>3m</th>
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<td>Polymorphic PVCs, managed with amiodarone and mexiletine</td>
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<td>Mean</td>
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<td>59.7±9.1</td>
<td>2.9±1.3</td>
<td>297.5±132</td>
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<td>46.6±7.9</td>
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M: Male, BMMNC: bone-marrow mononuclear cells, LVEF: left ventricular ejection fraction.
left ventricular function was performed together with radionuclide scanning in order to assess relative changes in segmental myocardial perfusion.

All values are presented as mean ± standard deviation. Changes in LVEF were analyzed with Wilcoxon signed ranks tests for related samples to detect statistical significance (IBM SPSS Statistics Version 19).

Results

All nine operated patients were male with a mean age of 59.7±9.1 years. Demographic and clinical data of all patients studied are presented in Table 1. LVEF calculated preoperatively ranged from 20% to 39% with a mean value of 31.3±6.5%. The mean number of grafted vessels was 2.9±1.3. One patient underwent concurrent mitral valve repair for functional mitral regurgitation. The mean volume of bone marrow that was aspirated was 297.5±132 ml. After processing the mean number of BMMNC that were isolated and injected into the myocardium was 32.3±24 x10^8 with a mean viability of 96%. This suspension contained a mixed population of stem cells. Flow cytometry analysis revealed that the subpopulation of CD34+ cells were 4.7±2.7 x10^6, CD105+ cells were 19.2±17.8 x10^6 while CD133+ cells were 20.1±30.3 x10^6.

There was no perioperative 30-day mortality. One patient died 5 months postoperatively due to acute gastrointestinal disease. The remaining 8 patients completed follow-up. One patient experienced polymorphic premature ventricular complexes (PVC) for a period of 6 months postoperatively managed successfully with amiodarone and mexiletine. Improvement was evident in LVEF which was increased significantly from 31.3% preoperatively to 42.4%, 46.6% and 52.5% at 3, 6 and 12 months respectively (Figure 1). On postoperative thallium scintigraphy increased perfusion in myocardial segments corresponding to areas of stem cell injection and a net reduction in the estimated infarct size at 6 and 12 months follow-up was evident in 5/8 (62.5%) patients (Figure 2).

Discussion

This study was designed as a safety trial in order to evaluate the effect of combined CABG and intramyocardial administration of autologous BMSC in pre-defined myocardial territories in patients with ICM. The procedure was well tolerated by all patients without any significant postoperative complications over a 12-month follow-up period. A significant improvement in systolic function of the left ventricle was observed with a gradual increase in LVEF from 31.3% preoperatively to 52.5% at 12 months.

After an episode of acute infarction the ischemic area becomes rich in inflammatory cytokines and protease activity, which also harms surrounding healthy cells. Thus, the initial localized injury creates a ripple effect that spreads slowly to larger areas of the heart. As the acute phase subsides granulation tissue develops as cardiomyocytes show very limited regenerative properties. In ICM the loss of functional tissue and subsequent remodeling eventually causes ventricular dysfunction and electrical instability leading to heart failure. Cellular therapy emerged as a potential adjunctive therapeutic strategy aiming to prevent remodeling by reconstitution of reperfusion, thereby leading to myocardial functional recovery. In our series of patients scar tissue was recognized as a dyskinetic or akinetic area on echocardiography. Thallium scintigraphy segmental analysis aimed to discriminate the non-viable scar tissue.
sue from the poorly perfused hibernating peri-infarct zone, which was the target zone for stem cell injection, when surgical revascularization was not considered feasible due to poor vessel quality. Optimal timing of cell therapy after acute myocardial infarction is debatable. Intracoronary administration can be performed in the early phases after the acute episode, while surgical intramyocardial delivery combined with coronary bypass grafting is associated with chronic ischemia.

Various cell populations have been investigated within the last few years in an attempt to find the best cell type for cardiac repair (autologous versus allogenic progenitor cells, skeletal muscle satellite cells, BMMNC, haematopoietic stem cells, mesenchymal stem cells and cardiosphere-derived cells). This disparity of results prevents scientific community from reaching solid conclusions regarding the optimal cell population that promotes myocardial reperfusion and potentially stimulate regeneration. We used a suspension of autologous BM-MNC which contained a mixed population of all different stem cells types that could be easily isolated and prepared while surgical procedure is ongoing obviating the need for isolation and culture of a specific cell type that would be more time consuming. Autologous bone marrow can be easily accessed through both anterior superior iliac crests, is renewable and does not produce an immune reaction. Bilateral anterior superior iliac crests offer the opportunity to aspirate larger volume of bone marrow compared to sternum. The mean volume aspirated was around 300 ml as concentration of stem cells in the final suspension is directly proportionate to the total volume obtained. The cell preparation protocol took 3 hours and the mean viability of cells was 96% without any contamination, which is indicative of high laboratory standards.

One of the most crucial and debatable methodologic questions in cellular therapy refers to the optimum method of cell delivery to the heart. The intracoronary delivery route has been tested in multiple clinical trials for cardiac repair after interventional revascularization of the coronary artery in the setting of acute myocardial infarction. Transendothelial passage and migration of stem cells into the infarct zone and subsequent engraftment into the host myocardium are considered questionable. Transendocardial delivery through a NOGA injection catheter placed to the target area combines intramyocardial cell delivery mimicking surgical technique while being less invasive. However, this method is technically demanding and carries the risk of potential complications. We used epicardial intramyocardial application into well-exposed myocardial areas which is associated with improved migration and homing of the transplanted cells into the host myocardium. Risk of ventricular arrhythmias after intramyocardial implantation of autologous BMMNC is not significant. There are recent reports of surgical stand-alone stem cell therapy through minimally invasive procedures which show promising results in enhancing myocardial reperfusion.

The mechanism of stem cell action in the diseased heart could be explained by two pathways; either stem cells directly transdifferentiate into functional cardiomyocytes or they exert a paracrine effect by secreting cytokines which increase residual myocyte viability and stimulate endogenous repair mechanisms through resident myocardial stem cells. Increasing evidence supports the hypothesis that paracrine factors that have antiapoptotic properties, promote angiogenesis and stimulate both residual normal cardiomyocytes and cardiac stem cells play the major role towards enhancement of myocardial perfusion and inversion of the remodeling process expressed as a reduction in the net infarct area as observed in our series of patients during serial follow-up with thallium scintigraphy.

There are certain limitations in the design of this study. It is a preliminary single-cohort study that lacks a control group. Global left ventricular function was assessed with echocardiography, while regional left ventricular function using cardiac magnetic resonance imaging (cMRI) would provide more accurate evaluation. Although there is a significant increase in perfusion and global contractility, the patient population is relatively small to reach a definitive conclusion regarding long-term outcome.

Conclusively preliminary results of a series of nine patients in our institution suggest that intramyocardial administration of BMSC in patients undergoing CABG for ICM is reportedly safe and associated with an improvement of left ventricular function and enhanced reperfusion of non-viable myocardial territories. In order to clarify the exact role of stem cell therapy and to delineate the mechanism of stem cell action into the host myocardium design of multicentre well-designed randomized trials including cell-labeling strategies is considered mandatory. Moreover, advances in cell processing technique could result in selection of subpopulations with improved homing and migration ability. Translational research could offer a realistic perspective towards a regenerative approach for chronic ischemic heart failure.

Conflict of interest
The authors disclose that there is no conflict of interest.

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