STEM CELLS TRANSPLANTATION AFTER LATE REPERFUSION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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ABSTRACT

Introduction: Experimental and clinical studies suggest that transplantation of bone marrow derived stem cells (BMC) beneficially affect left ventricular remodeling process after acute myocardial infarction (MI) and contribute to the regeneration and neovascularization of ischemic myocardium. Objective: To assess the safety and feasibility of autologous transplantation by intracoronary infusion of AC133⁺ BMC in patients with acute or recent MI. Methods: Patients with successful late reperfusion and stent implantation for acute or recent, large MI (LV ejection fraction (EF) <40%) were enrolled. AC133⁺ cells were transferred by balloon-catheter into the infarct-related artery (IRA). We assessed myocardial perfusion with myocardial Single-Photon Emission Computed Tomography (SPECT) (Technetium-99m-sestamibi) at baseline, 3, 6 and 12 months. Global LVEF change from baseline to 12 months follow-up was assessed by echocardiography (at 1, 3, 6 and 12 months). Nine patients were enrolled, with mean follow-up of 8±4 months; all completed 3 months follow-up. Results: No major periprocedural complications occurred. After 1 and 3 months all patients were free of angina and arrhythmias. After 3 months we have noticed significant decrease of perfusion defect detected by MIBI-scan (perfusion defect extent: 29.4±14.9% vs 36.3±12.3%, p<0.02) and significant increase in segmentary perfusion (38.6±15.2% vs 29.3±12.4%, p<0.01). Serial echocardiographic assessment demonstrated improvement of cardiac function at 3 months follow-up (EF: 40.5±8.9% vs. 33.7±5.4%, p<0.03). Conclusions: Our results suggest that autologous BMC transplantation by intracoronary infusion proves to be a safe and feasible method, with potential of myocardial function improvement.

Key Words: stem cells, myocardial infarction, autologous transplantation

INTRODUCTION

In patients with a large myocardial necrotic area resulting from acute MI, the loss of cardiomyocytes results in a fibrous tissue generation and aneurysm formation, in LV remodeling and subsequently, progression of congestive heart failure.¹ The necessity of regeneration of myocardial contractile cells pool has encouraged the development of alternative methods to classical medical therapies.² One of the most
promising strategies is represented by autologous cell transplantation with either differentiated cells (skeletal myoblasts, cardiomyocytes), or stem cells.

Recent research suggests that stem cells from whole bone marrow possess greater functional plasticity than was previously supposed. Since 1999, when Bittner et al. reported the possibility of cardiac muscle formation from circulating bone marrow cells, there have been many studies demonstrating stem cell contribution to cardiac muscle formation in both animals and humans. In adult mice with experimental MI, cardiomyocytes and vascular cells can be formed in vivo from circulating mouse BMC. Moreover, the stem cells also generate cardiomyocytes after direct injection into damaged heart tissue. In animal models, pluripotential cells from bone marrow improved myocardial function and perfusion in the setting of ischemic heart disease. In addition, recent publications have described beneficial effects (i.e. improvement in myocardial perfusion and segmental contractility) of autologous transplantation of mononuclear bone marrow cells in the immediate postinfarction period in humans.

All these data suggest that cardiomyocytes may be formed from bone marrow-derived hematopoietic and mesenchimal stem cells the extent of this process remaining to be clarified by further studies.

Therefore, the aim of our study is to investigate the safety and feasibility of autologous transplantation by intracoronary infusion of bone marrow–derived stem cells AC 133+ into the IRA in patients with acute and recent MI.

MATERIAL AND METHOD

Patient population

The patients were enrolled in this study after signing an informed consent approved by the Ethics Committee.

Inclusion criteria: age between 18-75 year, acute or recent MI (24 hours to 28 days), LVEF <40%, successful reperfusion – percutaneous coronary intervention (PCI) with stent implantation in IRA.

Exclusion criteria: age over 75 years, cardiogenic shock (systolic blood pressure < 80mmHg requiring intravenous pressors or intra-aortic counterpulsation balloon), a history of leucopenia /thrombocytopenia, renal failure with serum creatinine > 2.5mg%, evidence of malignant diseases, moderate/severe hepatic dysfunction, pregnancy, unwillingness to participate.

Baseline evaluation of the patients included a complete clinical assessment (history and physical), laboratory evaluation (blood count, ASAT, ALAT), electrocardiogram (ECG), and 48 hour Holter monitoring, transthoracic echocardiography (TTE), SPECT with Technetium 99 m Sestamibi. All patients received standard medical therapy at the time of enrollment.

The schedule of procedures in follow-up visits is summarized in Table 1.

Early after the procedure, ECG and 48 hours Holter monitoring were performed. Serum CK, CK-MB levels were assessed at 6 and 12 hours. Patients were monitored in the Cardiac Intensive Care Unit for 24 hours after the injection procedure.

Table 1. Follow-up time table

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical examination</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Lab</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>ECG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Holter monitoring</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>TTE</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SPECT</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>-Technetium</td>
<td></td>
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<td></td>
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<tr>
<td>99 Sestamibi</td>
<td></td>
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<tr>
<td>Coronary angio</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LV-angiography</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Bone Marrow Aspiration and Isolation of Stem Cells

A step by step scheme of the protocol concerning autologous stem cell from harvesting to injection is presented in Figure 1.

Bone marrow aspirates were obtained in the morning of the day of cell transplantation.
Approximately 12 hours before the cell injection procedure, bone marrow (500 - 700 ml) was aspirated from the posterior iliac crest under general anesthesia. Because of the high volume of bone marrow aspirate, patients with postprocedural haemoglobin below 10 g/dl received an iso-group transfusion (400 ml).

The cells were isolated by centrifugation, and the buffy coat was used. Buffy coat cells were incubated with 5% human immunoglobulin for intravenous use and AC133 beads (MACS) for 30 minutes, at 20°C. Then, the cells were washed with CliniMACS buffer 0.5% human serum albumin, centrifugated, and finally resuspended in the same buffer (final volume = 90 mL). This volume was introduced in the Clini MACS Plus magnetic cell sorter (Miltenyi Biotech, Bergish-Germany) for 90 minutes. Viability testing was assessed with trypan blue exclusion.

The quantitative analysis of AC133+ stem cells was performed by immunophenotyping, using a flow cytometer FACS Calibur with 2 laser (Beckton-Dickinson), Cell Quest acquisition software, and Paint A Gate software for data analysis. (Fig. 2, 3)

**Catheterization Procedure for Progenitor Cells Transplantation**

The procedure consisted in advancing an over-the-wire balloon catheter into the stent previously implanted during the reperfusion procedure.

To allow adhesion and potential transmigration of the infused cells through the endothelium, the balloon was inflated with low pressure to completely block blood flow for 3 minutes, while 3 to 5 ml of the progenitor cell suspension was infused distally to the occluding balloon through the central port of the balloon catheter. This maneuver was repeated 2 or 3 times to accommodate infusion of total 10 ml cell suspension, interrupted by 3 to 5 minutes of reflow by deflating the balloon to minimize severe ischemia.

After completion of intracoronary cell transplantation, coronary angiography was repeated to establish vessel patency and unrestricted flow of contrast material.

**Transthoracic Echocardiography**

A detailed resting two-dimensional and Doppler echocardiogram (with a Hewlett-Packard Sonos 5500 ultrasound equipment) was performed in all patients. Images taken from parasternal and apical windows were recorded on S-VHS videotape. LVEF was determined by a modified Simpson's method in the apical 4 and 2 chambers view.

**ECG gated myocardial SPECT with Technetium$_{99}$-Sestamibi**

A fasting state was recommended for 3-4 hours before the study. Patients underwent a rest study (Sestamibi 15 mCi); imaging commenced 60 minutes after intravenous injection of Technetium$_{99}$-Sestamibi.

Images were acquired using dual head gamma camera with a LEHR (low energy high resolution) collimator.

Reconstruction and pre-processing of the raw data were performed using filtered back-projection with a Ramp filter, and a Butterworth filter. The perfusion and functional data were analysed using a QPS/QGS protocol (Cedars-Sinai Medical Centre). Myocardial
outlines was divided into 20 segments representing the basal, mid and distal portions of the septal, anterior, lateral and inferior walls and the apex.

Each segment was scored at rest and stress using a five-point scoring system (0=normal; 1=equivocal; 2=moderate; 3=severe reduction of radioisotope uptake; 4=absence of detectable tracer uptake in a segment). The stress summed score (SSS) was calculated according with the extent and severity of perfusion defects at rest and during stress. The defect size was expressed in square cm and as a percentage of the left ventricle.

For segmentary perfusion assessment we considered only sections with perfusion <50% and for defect severity score were followed segments with severe reduction of radioisotope uptake (score ≥3).

**Angiographic analysis**

All the patients were part of the angiographic analysis (with COROSKOP TOP HI-P SIEMENS equipment), which involved three coronary angiograms (obtained at 3, 6 and 12 months follow-up). Angiographic follow-up was carried out to evaluate the patency of the target vessel and the status of native coronary arteries. Coronary angiograms were obtained according to standard acquisition guidelines.

**Follow-up**

At follow-up visits (1, 3, 6 and 12 months) all patients underwent a complete clinical assessment. ECG monitoring for 48 hours and cardiac echocardiography were recorded. Coronary angiography, LV-angiography and positron emission tomography were performed 3, 6 and 12 months after implantation in all patients.

**Statistical analysis**

The statistical methods are based on the common biometrics methods commonly used in the analysis of clinical events.

The data were analyzed according to the following criteria:

-continuous variables were expressed as mean ± standard deviation (SD);
-discrete variables were expressed as counts and percentages of the study population;

Comparison of the perfusion defect size and EF from baseline to 1 and 3 months follow-up were made with paired t-test.

For all tests, a p value < 0.05 was considered significant.

**RESULTS**

The demographic, clinical and angiographical characteristics of patient population are presented in Table 2.
aspirated from the posterior iliac crest during a brief general anaesthesia. No bleeding complications at the harvest site were noted. The final preparation of bone marrow AC133+ cells contained 5.3 ± 1.7x10^6 AC133+ (the positive fraction oscillated from 4.3x10^6 to 8x10^6 AC133+ stem cells), cells with purity 92.26 ± 3.67% and viability 97 ± 1%. (Table 3)

**Table 3. Characteristics of bone marrow cells injected**

<table>
<thead>
<tr>
<th>Bone marrow cells</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow aspirate (ml)</td>
<td>605.56 ± 88.2</td>
</tr>
<tr>
<td>AC 133+ Purity (%)</td>
<td>92.26 ± 3.67</td>
</tr>
<tr>
<td>AC 133+ Viability (%)</td>
<td>97 ± 1</td>
</tr>
<tr>
<td>AC133+ Injected</td>
<td>5.3 ± 1.7x10^6</td>
</tr>
</tbody>
</table>

One patient had signs of clinical worsening after cell transplantation procedure (transient worsening of heart failure signs and symptoms which necessitated discontinuation of beta-blocker therapy, mechanical circulatory support with intraaortic balloon counterpulsation and intravenous positive inotropic drugs). No other major periprocedural complications occurred (no significant changes in serum levels of CK, CK-MB) and no malignant arrhythmias were found on 48-hours Holter monitoring. All patients were free of angina during hospitalization. Patients were discharged after 13.6 ± 6.6 (7-28) days of hospitalization.

At 3 months follow-up visit all patients were free of angina and no signs of clinical worsening were noticed. No malignant arrhythmias were recorded on 48-hours Holter monitoring and no pericardial effusion was detected on echocardiography. At 3 months coronary angiography a new significant lesion was detected at the proximal stent edge in the target vessel and was treated by direct stenting procedure. In two cases angiographic analysis revealed at 3 months follow-up visit LV aneurism. In one of the patients, SPECT analysis detected a 9.7% reduction of perfusion defect extent compared to baseline; the aneurism had stationary appearance at 12 months and the patient was referred for surgical correction. The other patient presented on MIBI scan analysis LV dilatation with increase of perfusion defect extent. Third grade ischaemic mitral regurgitation was diagnosed in this case on baseline and 3 months follow-up visit, with possible implication in this unfavorable LV remodeling process.

After 1 month LVEF showed no significant change compared with that before BMC transplantation (35.2 - 8.2% vs 33.7 ± 5.4%, p<0.22), but increased significantly after 3 months follow-up (40.5 - 8.9% vs 33.7 ± 5.4%, p<0.03). (Fig. 4, 5)

At 3 months follow-up perfusion defects were reduced with 14%, and defect severity score diminished from 6.1 ± 2.5 to 5.2 ± 2.5 (Fig. 6).

![Figure 4. Global ejection fraction (TTE, 2D)](image1)

![Figure 5. Global ejection fraction (mean ± SD) (TTE, 2D)](image2)

![Figure 6. Defect severity score detected by SPECT (mean ± SD)](image3)

![Figure 7. Perfusion defect extent detected by SPECT (mean ± SD)](image4)
Perfusion defects detected by positron emission tomography decreased significantly after 3 months compared with baseline (29.4 ± 14.9% vs 36.3 ± 12.3%, \( p < 0.02 \)) (Fig. 7) and segmentary perfusion was improved (38.6 ± 15.2% vs 29.3 ± 12.4%), (Table 4).

**Table 4. Cardiac indexes at three-months follow-up**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>3 mo follow-up</th>
<th>Change</th>
<th>( p ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF by TTE (%)</td>
<td>33.7 ± 5.4</td>
<td>40.5 ± 6.9</td>
<td>6.7 ± 6.4</td>
<td>(&lt;0.03)</td>
</tr>
<tr>
<td>Perfusion defect by SPECT (%)</td>
<td>36.3 ± 12.3</td>
<td>29.4 ± 14.9</td>
<td>6.8 ± 4.7</td>
<td>(&lt;0.02)</td>
</tr>
<tr>
<td>Segmenary perfusion by SPECT (%)</td>
<td>29.3 ± 12.4</td>
<td>38.5 ± 15.2</td>
<td>9.4 ± 11</td>
<td>(&lt;0.01)</td>
</tr>
<tr>
<td>Defect severity score by SPECT</td>
<td>6.1 ± 2.5</td>
<td>5.2 ± 2.5</td>
<td>-0.9 ± 1.3</td>
<td>(&lt;0.0004)</td>
</tr>
</tbody>
</table>

We observed a significant reduction in MIBI scan determined infarct size (10% reduce of perfusion defect extent compared to baseline) in 6 patients, with >20% reduction of perfusion defect extent in 4 of them. (Fig. 8)

**DISCUSSIONS**

The ability of stem cells transplantation to regenerate infarcted myocardium and beneficially affect ischaemic myocardium was shown to have considerable therapeutic potential by several groups.\(^{11-26}\)

For the moment it is unknown whether stem cells therapy would be more beneficial in the early infarction or in its later remodeling phase, or even more valuable in the end-stage of ischemic cardiomyopathy.\(^{27}\)

The previous studies refer to clinical trials in both early and late stages of acute MI or in patients with severe ischaemic heart disease which are not suitable for complete revascularization procedures (poor distal vessels, unacceptable procedural risks) and are refractory to maximum medical therapy.

Stem cells were delivered by intracoronary injection into the IRA, direct injection into heart muscle at the time of coronary artery bypass grafting (CABG) or by direct transendocardial injection.\(^{11-26}\) The cells used in these trials were different, from unfractioned bone marrow cells to various populations of BMC; there is no data regarding the benefits of using a specific stem cell subpopulation. Moreover, it is not clear whether these cells can function normally when are surrounded by scar tissue, or whether angiogenesis is a critical component of a successful myocardial regeneration.\(^{28}\)

After stem cells therapy during the early postinfarction period an improvement in LV function was reported, involving a significant increase in myocardial perfusion with reduction in infarct size and beneficial effects on LV remodeling process.\(^{11-18}\) In patients with advanced coronary artery disease, with no-options for complete revascularization, stem cells therapy was associated with improvement of clinical status and myocardial perfusion and function.\(^{18-25}\)

Most of these studies have reported few side-effects. One of them consisted of intrastent restenosis which determined the early termination of one study using treatment with granulocyte-colony stimulating factor and intracoronary infusion of collected peripheral-blood stem cells.\(^{17}\) Acute myocardial ischaemia and subacute myocardial microinfarctions has been reported in dogs that underwent intracoronary infusion of mesenchymal stem cells.\(^{29}\)

Mesenchymal stem cells are also present in the fraction of cells administered to patients in most studies; one study which used autologous bone marrow mesenchymal stem cells for intracoronary injection in patients who underwent primary PCI within 12 hours after onset of acute MI has not documented an acute adverse event.\(^{16}\)
Few small randomized trials have directly tested the hypothesis that mechanical opening of persistent occlusions late after MI (more than 12 hours to days after coronary artery occlusion), when salvage of ischemic myocardium is unlikely, will improve long term LV remodeling and clinical outcomes (the late open-artery hypothesis).30,34 Most studies showed no significant changes in LV size and function in PCI-group therapy. Coronary patency obtained in the first 12 hours after MI (PCI with stenting) has been associated with increasing of LVEF after 6 months (primarily attributable to lower rates of early and late restenosis and reocclusion of the IRA).35,36 Sheiban et al. have shown that revascularization of the IRA by angioplasty to more that 4 hours from onset of symptoms will not provide significant improvement of LVEF.37 In our study all patients have undergone late reperfusion of infarct related coronary artery and we can presume that 6.7 ± 6.4% increase in LVEF (which is comparable to that observed in other clinical trials) can be attributable to stem cells therapy.11,13,15 The benefits of the cell transplantation was observed both in patients with moderate impairment of LV function and in those with more important depression of LV function at the time of their acute episode.

The stem cells compartment in human bone marrow is highly complex, comprising both CD34+ and CD34- hematopoietic stem cells, mesenchymal progenitors, and perhaps other cell types whose activities remain to be defined. We used AC133+ cells, which include a non-hematopoietic (CD34) subpopulation of BMC that have a high potential to induce angiogenesis compared to unselected bone marrow cells.38 We selected this type of stem cells because we hypothesized that angiogenesis was the most important mechanism responsible for improvement in myocardial function in our patients. Moreover, this population, called mature adult progenitor cells (MAPC) was demonstrated to differentiate to both visceral mesoderm cells, such as endothelial cells, and cells of limb-bud mesoderm, including myocytes. Therefore, using AC133+ cells might ensure both replacement of damaged myocardium and improvement of perfusion by stimulating neangiogenesis. Of course, it is very probable that a complex sequence of events that includes not only the presence of the transplanted cells, but also the action of cytokines and growth factors, and intricate cell-to-cell interactions, may all contribute to the final result.39

Another important aspect concerns the homing process, which results in cell engraftment. After acute events, serum vascular endothelial growth factor (VEGF) levels rises significantly, and it is expected that homing signals may be more powerful in acute and subacute ischemic syndromes.40 This was one of the reasons that determined us to choose patients with acute or recent MI.

Autologous transplantation by intracoronary infusion of bone marrow-derived mononuclear cells (into the IRA) was associated with significant increase of LVEF in TOPCARE-AMI and BOOST trial.11,13,15 Transplantation of progenitor cells reduced infarct size with favorable effects on LV remodeling process and lack of infarct expansion (TOPCARE-AMI). Both trials recruited patients with reperfused acute MI-PCI at 27 ± 40 respectively 2 - 22 hours from symptom onset. We used a different subpopulation of bone-marrow cells (AC133+) in patients with recent or acute MI reperfused after 12 hours. The same benefits were observed but two cases have developed LV aneurism with indication for surgical correction.

Our results are also comparable to those described by Stamm et al. who delivered AC133+ stem cells in 6 patients who had had an acute transmural MI older than 10 days, but less than 3 months before admission, and were candidates for CABG.36 They reported good clinical results after 3-9 months after surgery. All patients survived, with a significant improvement of perfusion in the previously non-perfused or hypoperfused infarct zone in 5 patients, and an enhanced global LV function in 4 patients. In our study, we aspirated larger amounts of bone marrow from the iliac crest (600 – 700 ml, versus 85 – 195 ml in Stamm’s trial) in order to isolate a higher number of AC133+ cells. We preferred to deliver more AC133+ stem cells (4.5-8x10⁶ cells, compared to 1.21-3.37x10⁶ cells in Stamm’s trial), in order to be sure that a higher number of cells will remain in the myocardium, but we have not remarked a direct relation between the number of delivered cells and benefits.

Homing signaling may not be as intense in patients with chronic ischaemic heart disease and therefore, might not be optimal for cell engraftment in these patients. Transendocardial injection of stem cells using electromechanical mapping to identify viable myocardium offers a theoretical benefit over surgical or intracoronary approaches.21 There is data which suggest the relative safety of this procedure, underlining that this alternative procedure improved both myocardial perfusion and function of the ischaemic region on cardiac MRI.20,21

The major limitations of this study are the absence of the control group, the small number of patients enrolled and the lack of appropriate methods to
disclose the mechanism of myocardial tissue repair and functional improvement after AC133+ cells transplantation.

CONCLUSIONS

The preliminary results of our study suggest that intracoronary injection of AC133+ cells in patients with acute and recent MI is safe and feasible, with potential of myocardial function improvement. Transplantation of AC133+ BMC was associated with significant decrease of perfusion defect and improvement of LV function.

REFERENCES