Stem Cells Implant in the Heart. Outcomes at 43 months

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SUMMARY

Background
Heart failure following a myocardial infarction is one of the major problems a cardiologist has to deal with. The reduction in the number of cardiac myocytes with subsequent formation of unfunctional fibrotic scars produces irreversible damage for cardiac function. Recent studies have demonstrated that implant of bone marrow stem cells in the myocardium offers a new possibility to recover systolic function after myocardial infarction. These preliminary data suggest the feasibility of implanting bone marrow stem cells in patients with dilated cardiomyopathy secondary to coronary heart disease.

Objectives
To assess the feasibility and safety of bone marrow stem cells implant in the heart of patients with ventricular dysfunction secondary to necrotic scars, and to evaluate changes in clinical symptoms and segmental wall motion of areas of non-viable tissue during long-term follow-up.

Material and Methods
We included 13 male patients (mean age 53.6±10.3 years) with an average follow-up of 43 months. A total of 88 segments had fibrotic tissue (6.77 segments per patient). Concomitant coronary artery bypass graft surgery was performed in remote ischemic and viable areas. Patients were evaluated with dobutamine stress echocardiography, color kinesis and radionuclide ventriculography.

Results
The cell suspension contained 0.7%±0.4% CD34+ cells. Cell viability was greater than 95%. Stem cells were implanted by 33.07±8.2 injections during coronary surgery via sternotomy with an average of 5.93±2.2 ml of solution in akinetic and metabolically non-viable segments. Adverse outcomes were not reported during hospitalization. Mean New York Heart Association functional class improved from 2.4±0.5 to 1.1±0.3 (p<0.0003), the ejection fraction increased from 26.4%±8.6% to 34.6%±13% (p<0.001). Left ventricular diastolic diameter did not vary during follow-up. Postoperative tests, performed by independent observers, demonstrated functional recovery in 47% of the segments implanted. Four patients died during follow-up: three of extracardiac causes and one of heart failure.

Conclusions
These findings demonstrate feasibility and safety in the implantation of stem cells. The recovery of non-viable segments suggests functional efficacy at long-term follow-up; however, further controlled studies are necessary to confirm these results.

Key words
Cellular Cardiomyoplasty - Stem Cells - Heart Failure - Myocardial Infarction
Until this moment, there is lack of clinical research strategies focused on solving the question whether cell implants modify the viability in a fibrous scar. In particular, the selection of a model of acute ischemia reunites too many variables of synchronous action which are impossible to be assessed independently. These variables cloud the analysis of the use of bone marrow stem cells (e.g., reperfusion due to angioplasty, spontaneous recirculation in the injured zone, collagenolytic activity of matrix metalloproteinases, cell implantation). (10, 11) We believe that most studies focus their search on functional outcomes that are far from the initial step of organic viability that should be demonstrated by the most possible pure models.

On the contrary, in the chronic fibrotic model used in this investigation, the strict analysis of the efficacy of the outcomes should be related with the basic goal of the study: to observe the changes produced by implantation of cells in nonviable areas metabolically inactive and with nonrevascularizable lesions. In this sense, it is essential to perform studies to evaluate the contractile status of the segments treated with cell implant, as these patients undergo concomitant revascularization surgery. This constitutes a limitation in this model, even in the presence of isolated ischemia in areas remote to the grafted scars.

The goal of this investigation is to assess the feasibility and safety of bone marrow stem cells implant in the heart of patients with ventricular dysfunction secondary to necrotic scars, and to evaluate changes in clinical symptoms and segmental wall motion of areas of non-viable tissue during long-term follow-up.

MATERIAL AND METHODS

Inclusion criteria
Patients were included in the basis of the following criteria:

a) Left ventricular systolic dysfunction with an ejection fraction < 40% calculated by echocardiography and radionuclide ventriculography.

b) History of myocardial infarction with presence of non-viable tissue scars demonstrated by two different methods.

c) Ventricular wall thickness > 5 mm.

d) Indication of concomitant coronary artery bypass graft surgery in a viable or ischemic remote area (different from the transplanted area) with a coronary artery anatomy unsuitable for percutaneous coronary intervention.

e) Functional class (NYHA) > II.

Exclusion criteria

a) Unmanageable arrhythmias.

b) History of myocardial infarction < 4 months.

c) Positive virus test results (HIV, cytomegalovirus, hepatitis B and C).

d) Surgical urgency.

e) Pregnancy.

f) Severe conditions; concomitant neoplasms or infections.

Preoperative assessment

All patients underwent Doppler-echocardiography, radionuclide ventriculography, coronary angiography and left ventriculography. Myocardial viability was determined by rest-dipyridamole gated SPECT with 99mTc-sestamibi and dobutamine stress echocardiography. The analysis of baseline segmental wall motion was performed using ultrasound scanners SONOS 2500 and SONOS 5000 with color kinesis and fusion harmonics, respectively. Images were interpreted using a 17-segment heart model and each myocardial segment was assigned a wall motion score according to the American Heart Association guidelines. (12) The results of the echocardiograms were analyzed by two different specialists; non-viable segments were considered only in case of agreement. The postoperative evaluation was performed by the same specialists who were not aware of the segments undergoing implantation.

Population
From April 2003, our experience included 13 male patients (53.6 ± 10.3 years). All patients had ventricular dysfunction secondary to myocardial infarction and indication of myocardial revascularization of remote areas. A total of 88 segments had fibrotic tissue (67.7 segments per patient). Hypokinetic segments were excluded from the analysis in order to make the protocol more rigorous, avoiding the possibility of considering that any improvement of hypokinetic segments might be due to the surgical revascularization rather than to cells implant.

All patients had symptoms of heart failure in functional class II-III and evidence of myocardial ischemia in the territory of the left anterior descending coronary artery demonstrated by the presence of angina, and by functional echocardiography and radionuclide tests. The remaining coronary arteries were occluded and had nonrevascularizable lesions.

The average preoperative data of these patients were as follows: NYHA functional class was 2.4 ± 0.5; left ventricular ejection fraction was 26.4% ± 8.6% and left ventricular diastolic diameter was 63.7 ± 8.8 mm (Table 1).

Myocardial revascularization surgery and cell implant
About 100 ml of bone marrow aspirate was harvested from iliac crest 6 hours before surgery. In the meantime, the sample was processed and enriched. All patients underwent surgical revascularization using internal mammary artery grafts to the left anterior descending coronary artery without cardiopulmonary bypass, to avoid the addition of perfusion variables that might result in invalid outcomes. After myocardial revascularization ended, the cells were implanted within and around the scars. An average of 152 ± 78.9 × 10⁶ cells, in an solution of 5.93 ± 2.2 ml (range 2.9-8.4) containing a average preoperative data of these patients were as follows: NYHA functional class was 2.4 ± 0.5; left ventricular ejection fraction was 26.4% ± 8.6% and left ventricular diastolic diameter was 63.7 ± 8.8 mm (Table 1).

Statistics
The results are expressed as mean ± standard deviation. Preoperative and postoperative results were compared using the Student’s t test; a p value < 0.05 was considered statistically significant. The study protocol was approved by the Committee on Ethics of our institution. All patients received detailed information and gave their consent.

RESULTS

Immediate postoperative period
Neither bone marrow aspiration nor coronary artery bypass graft surgery affected the outcomes of bone marrow stem cells implant. No malignant arrhythmias...
or deaths were reported. All patients were discharged between days 7 and 15 after the procedure.

Follow-up
Total follow-up was 17504 days with an average of 1346 ± 590 days/patient, or 43 months. Four patients died: 3 due to non-cardiac causes (esophageal cancer, traffic collision, stroke) and 1 due to heart failure at 248, 1010, 1937 and 1120 days, respectively. No implant-related complications were reported.

NYHA functional class improved from 2.4 ± 0.5 to 1.1 ± 0.3 (p < 0.0003). Left ventricular ejection fraction increased from 26.4% ± 8.6% to 34.6% ± 13% (p < 0.001); left ventricular diastolic diameter presented a non-significant reduction from 63.7 ± 8.8 to 61.4 ± 3.8 mm (Table 1).

In addition, 42 segments out of the initial 88 (47%) fibrotic segments presented recovery of wall motion abnormalities (Figure 1). Nonviable segments per patient decreased from 6.77 to 3.5 (Table 2).

Figure 1 shows an example of recovery of wall motion abnormalities in fibrotic segments studied with 99mTc-sestamibi.

DISCUSSION
When we started the clinical phase of this investigation, we remained faithful to a model that avoided cardiopulmonary bypass, the likelihood of systemic inflammatory response, and multiple revascularizations. In addition, we only considered akinetic and dyskinetic segments for the analysis. However, hypokinetic segments underwent concomitant stem cells implant and myocardial revascularization surgery but were not included in the analysis, as these segments are more likely to improve with revascularization surgery. We know that less than 10% of nonviable segments may improve with isolated myocardial revascularization surgery. (1) In addition, fibrotic segments have less than 25% of viable cardiomyocytes. At least 50% of viable myocytes are required to achieve a successful revascularization; (1) thus, it is possible to consider that the change in the viability of dyskinetic and akinetic segments may be due to cell implant.

We believe that the experience accumulated along the last five years has demonstrated some aspects of an image that is waiting to be revealed. In a world in which diversity is a fundamental feature, man tends

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Table 1. Cell implant. Preoperative period and 43-month follow-up (n = 13).

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<td>Total</td>
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Table 2. Recovery of segments per patient
to claim for unity and coherence; however, we should feel satisfied with partial and provisional results. In this sense, and beyond some unknown issues, our own experience makes us see signs of therapeutic efficacy in terms of functional capacity and ventricular function, as well as positive changes in myocardial viability. In our own experience with myoblasts, we have seen that angina-free patients before the procedure may develop angina during follow-up due to tissue recovery as, basically, myoblasts produce myogenesis and stem cells angiogenesis. (5) The analysis of segmental wall motion before and after the procedure has been extremely important to evaluate the outcomes. NYHA functional class improved from 2.4 ± 0.5 in the preoperative period to 1.1 ± 0.3 after surgery (p < 0.0003) and ejection fraction increased from 26.4% ± 8.6% to 34.6% ± 13% (p < 0.001). Postoperative echocardiography and radionuclide studies, performed by independent observers, demonstrated functional recovery in 47% of the segments implanted. Nonviable segments decreased from 6.77 to 3.5.

The use of SPECT, PET or MRI techniques are appropriate to evaluate changes in viability in acute models (acute ischemia) and chronic models (fibrotic myocardium, dilated cardiomyopathy, Chagas disease), especially in patients with previous myocardial infarction. The presence of changes in the viability of the compromised segments means the step forward in this field. Possibly, new scores should be developed to evaluate the minimal changes in viability produced by stem cells implant.

The protocol of the University of Navarra uses G-SCF (growth factor of granulocytic colonies) as inductor during five days, and later the cell fraction AC133 is isolated by column plasmapheresis. Contrary to the experience of Zohlnhöfer et al, (13) who only used G-SCF, we should mention that G-SCF has not proved to be more effective than the recruitment of proangiogenic cells, as this method ensures the necessary number of cells in the concentrate. There is no certainty either that these mobilized stem cells home to the adequate place in a sufficient percentage to avoid the necessity of direct placement of cells. Efforts have been made in an attempt to find answers applicable for clinical practice, based on basic and applied research. The lack of favorable outcomes in the study by Zohlnhöfer et al. (13) are related to the type of cells and the route of administration used, probably a bad influence of the methodological chaos that is present in the different reports, which needs self-organization.

Janssens et al. (9) performed a randomized, double-blind, placebo-controlled study with bone marrow derived stem cell transfer in patients with ST-elevation acute myocardial infarction. Yet, some ethical aspects should be considered. Is it feasible to harvest bone marrow from patients, perform a coronary angiography and inject saline solution into the coronary arteries of control patients with acute myocardial infarction? We believe that the health care market is moving towards science progress without considering the ethical issues, despite the authors find this experience crucial.

Patients included in this study constituted a low-risk population with 38% of the infarctions in the territory of the right coronary artery and an ejection fraction of 55%, a value not likely to experience significant changes. Cells were transferred 24 h after percutaneous coronary intervention and cannot, therefore, exclude potential activity of matrix metalloproteinases, a disadvantage for the survival of the

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Fig. 1. Gated SPECT with 99mTc-sestamibi showing changes from cell implant to 5-year follow-up.
injected cells. Although cell therapy did not produce significant improvement, it could favorably affect infarct remodelling.

Meyer et al. (10) analyzed the outcomes after 18 months of follow-up of the BOOST trial, which included patients who underwent transfer of autologous bone marrow cells after an acute myocardial infarction. The original study was published by Wollert et al. (11) who found that transfer of autologous bone marrow cells promoted improvement of left-ventricular systolic function in patients after 6 months. After 18 months, LV ejection fraction increased by 3.1% in the control group and 5.9% in the bone marrow cells transfer group; this difference was not significant and is consistent with our previous analysis in relation with maintaining the performance.

Basic research and clinical trials should be carried out to understand the unrevealed intelligibility of nature in the stem cell field.

CONCLUSIONS

These preliminary data suggest the feasibility of implanting bone marrow stem cells in patients with dilated cardiomyopathy secondary to coronary heart disease. (14, 15) The recovery of non-viable segments suggests functional efficacy in this sample. Further large randomized studies are necessary to confirm these conclusions. (16) In a near future autologous transfer of bone marrow cells may be a useful resource in daily practice for tissue regeneration in patients with dilated cardiomyopathy.

BIBLIOGRAPHY