BRIEF COMMUNICATION

Myoblast Transplantation in the Heart: Outcomes at 5-Year Follow-Up

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ABSTRACT

Several centers all around the world, including some institutions in Argentina, performed the first experiences in clinical phase with myoblast transplantation for cardiac regeneration. These initial studies were published during 2002 and 2003. The outcomes of these patients at 3-year follow-up were reported thereafter. It is in our interest to present the outcomes of the same group of patients 5 years after the implant.
We have classified the compromised segments in 4 categories: transmural infarction, nontransmural infarction, ischemic and normal. In this way, 68 segments from 4 patients who were alive at 66±6.2 months were available for evaluation. There was a clear reduction in the number of segments with transmural infarction and an increase in the number of segments with nontransmural infarction and with ischemia. The number of segments with transmural compromise decreased from 15 to 3 (risk reduction of 80%; p=0.0005). Analysis of nontransmural segments should be performed in a comprehensive fashion. The global number of these segments increased from 7 to 9; however, preoperative nontransmural segments originally reported decreased from 7 to none. It might be considered that the global increase of these nontransmural segments corresponded to segments incorporated not only as a consequence of the progression of the disease but also at the expense of transmural segments, in a clear reduction of the fibrotic tissue.

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Key words > Myoblasts - Cells Transplantation - Heart Failure

Abbreviations >

FC	Functional class	**LVEF**	Left ventricular ejection fraction
LVDD	Left ventricular diastolic diameter		

BACKGROUND

The replacement of cardiac muscle by fibrotic tissue is a well-known mechanism. Loss of muscle mass may be partially compensated by cellular hypertrophy; however, remodeling leads to progressive congestive heart failure. Understanding the different disciplines derived from genetics and cell biology has enabled to establish of a project of cell self-repair. Nevertheless, there are still some issues to elucidate before this possibility becomes a feasible therapeutic option. Has the procedure proved to be efficient? Which is the best cell to use? How should cells be implanted? These are some of the questions to solve. This technique should be based on long-term outcomes to reach levels of clinical feasibility and indices of therapeutic reliability.

Several centers worlwide, including some institutions in Argentina, performed the first experiences in clinical phase with myoblast transplantation for cardiac regeneration. (1-10) These initial studies were published during 2002 and 2003. (8-10) The outcomes of these patients at 3-year follow-up were reported thereafter. (11) It is our interest to present the outcomes of the same group of patients five years after implantation. The aim of this communication is to report the outcomes at 66 ± 6.2 months of myocardial segments with transmural necrosis that underwent myoblast implantation. Analyses of survival or functional variables are beyond the scope of this report. The original studies from each patient were entirely revaluated.

POPULATION

Five patients had been included in the first report (8) in 2003; 13 months later, one patient died due to congestive heart failure secondary to a new myocardial infarction. Therefore, the patient did not complete

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follow-up and was excluded from the analysis. This report includes the remaining 4 patients who survived: they were men, mean age 61 ± 6.4 years (range: 52-66), with ventricular dysfunction secondary to myocardial infarction and with indication of myocardial revascularization of remote areas. Infarctions were located in the inferoposterolateral wall (n = 2), inferior wall (n = 1) and posteroinferior wall (n = 1). In all cases the culprit coronary vessels were occluded and had nonrevascularizable lesions.

All patients had symptoms of heart failure in functional class (FC) II, evidence of myocardial ischemia in the territory of the left anterior descending coronary artery (the only vessel that had undergone revascularization), demonstrated by the presence of angina and/or by functional tests, echocardiography, radionuclide cardiovascular imaging or refractory angina.

Stress and rest Tc-99m sestamibi gated SPECT images were interpreted using a 17-segment heart model. A grading scale of 0 to 4 was used to score perfusion in each segment, where 0 = normal perfusion, 1 = mild hypoperfusion, 2 = moderate hypoperfusion, 3 = severe hypoperfusion, and 4 = absence of perfusion.

A score of 4 or 3 during exercise and rest, with absence of wall thickening in gated images identified non-viable transmural segments (transmural infarction). Absence of viability is non-transmural (non-transmural infarction) when perfusion score at rest changes to 2 or 1.

An average of 200 ± 47 × 10^6 cells, in an solution of 3,9 ± 1,4 ml (range 3-6) containing a percentage of CD56+ cells (myoblasts) of 54% ± 8,6% were implanted by 22 ± 8 injections (range 10-27).

STATISTICS

Continuous variables were expressed as mean ± standard deviation (SD) and were compared with t test, while discrete variables were compared with the chi square test. A p value < 0.05 was considered statistically significant.

Follow-up

All patients are in FC I after a mean follow-up of 66 ± 6.2months (range 61-75 months). Left ventricular ejection fraction (LVEF) increased from basal values of 36% ± 6.1% to 45% ± 13.4% (p = ns) after myoblasts transplantation, and a reduction in left ventricular diastolic diameter from 61.7 ± 4.6 mm to 60,9 ± 6.3 mm (p = ns) also occurred. Tables 1 and 2 and Figure 1 illustrate the changes found in each segment.

DISCUSSION

Myoblasts used in this research come from satellite cells of skeletal muscle tissue. These cells reproduce easily and have been widely studied; for this reason they are used in clinical phase. Satellite cells lie in a

Table 1. Transmural, non-transmural (nonT) and ischemic segments. Changes at 6 and 33 months

		Viable				
	T	Non T	Ischemics	Normal	New	Total
Pre	6+4+3+2 15	1+0+3+3 7	0+2+0+0 2	10+11+11+12 44		68
6 months	2+4+1+1 8*	3+0+2+2 7	0+0+0+1 1	12+13+14+13 50	0+2+0+0 2	68
33 months	2+1+0+0 3#	3+3+2+2 10	1+0+2+1 4	11+9+13+12 45	0+4+0+2 6	68
66 months	2+1+0+0 3#	3+2+2+2 9	0+0+0+3 3	12+12+15+12 51	0+2+0+0 2	68

T: Transmural segments. NonT: Nontransmural segments. I: Ischemic segments. Pre: Preoperative.
Numbers on top indicate the amount of segments in each of the 4 patients.
The number on the bottom indicates the total segments in the 4 patients.
* p = 0.0082 versus pre.
* p = 0.0005 versus pre.

Table 2. Changes in transmural segments per patient at 33 months.

	Pre	Viable		Unchanged	
Patients	T	Non T	Ischemic	Normal	T
1	6	3	0	1	2
2	4	2	0	1	1
4	3	2	0	1	0
5	2	2	0	0	0
Totales	**15**	**9**	**0**	**3**	**3**

T: Transmurales. No T: No transmurales.

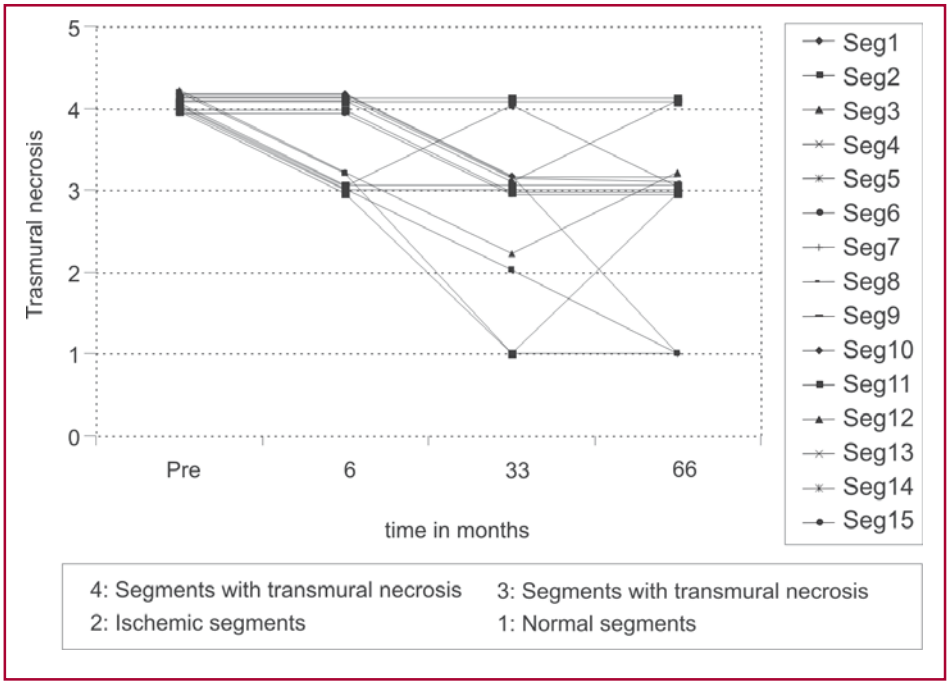

Fig. 1. Changes in segments with transmural necrosis.

quiescent state under the basal membrane of mature muscular fibers until a stimulus triggers their replication. Myoblasts are precursor cells of the mesoderm that are destined for myogenesis of heart, skeletal and smooth muscle tissue.

These cells have high resistance to ischemia and are implanted in necrotic areas which are never completely avascular, where they grow and replicate. The main goal of this study was to demonstrate the feasibility of this technique in humans, supported by a wide international experience in several animal species. Clinical outcomes were favorable, as we demonstrated that myoblasts replicate from the initial biopsy until achieving a large number of cells for transplantation after 3 to 4 weeks of cell culture.

The procedure was safe and no complications during surgery or follow-up were reported. In this sense, the capacity of myoblast oncogenic proliferation or migration to osteoclasts or chondroblasts is low. One of the advantages of myoblasts is the wide range of phenotypic possibilities of the myogenic lineage, compared to bone marrow stem cells.

Traditional cell culture techniques use fetal bovine serum for cell growth. However, contact of human cells with fetal bovine serum after three weeks results in fixation of animal proteins on the cell surface, representing an antigenic substrate for immunological and inflammatory adverse events, as observed in Menasché's experience. (1) We have been using autologous human serum for cell culture since the beginning of the trial. We were the first researchers to use this type of culture in a clinical trial as it has been well recognized and demonstrated that it does not produce arrhythmias. (12, 13)

The mechanisms related to improvement of heart performance with cells transplantation are controversial. Several direct and indirect factors contribute to structural and functional benefits. Implantation of muscle cells might increase regional elasticity, modifying cellular matrix and improving ventricular remodeling. We have recently published our clinical experiences adding a collagen matrix seeded with bone marrow cells. (14)

This study was only designed to analyze changes produced by implantation of myoblasts in nonviable areas metabolically inactive and with nonrevascularizable lesions (Figure 2). In this sense, it is essential to perform studies to evaluate the contractile status of the segments treated with cell implantation, as these patients underwent concomitant coronary artery bypass grafting. Although this constitutes a study limitation, patients only presented anteroseptal ischemia, remote to the grafted scars.

Patients remained in FC I after 3 and 5 years of follow-up. No significant differences were observed in LVDD at basal (61.7 ± 4.6 mm) and at 3 and 5-year follow-up (59 ± 5.8 mm and 60.9 ± 6.3mm, respectively). Left ventricular EF increased from 36% ± 6.1% to 46% ± 12.2% 3 years after the procedure ($p < 0.05$). Nevertheless, there were no significant differences at 5 years (45% ± 13.4%) Global analysis revealed clinical stability according to the variables evaluated.

We have classified the compromised segments in 4 categories: transmural infarction, nontransmural infarction, ischemic and normal. In this way, in 68 segments from 4 patients who were alive at 66±6.2 months we found a clear reduction in the number of segments with transmural infarction and an increase

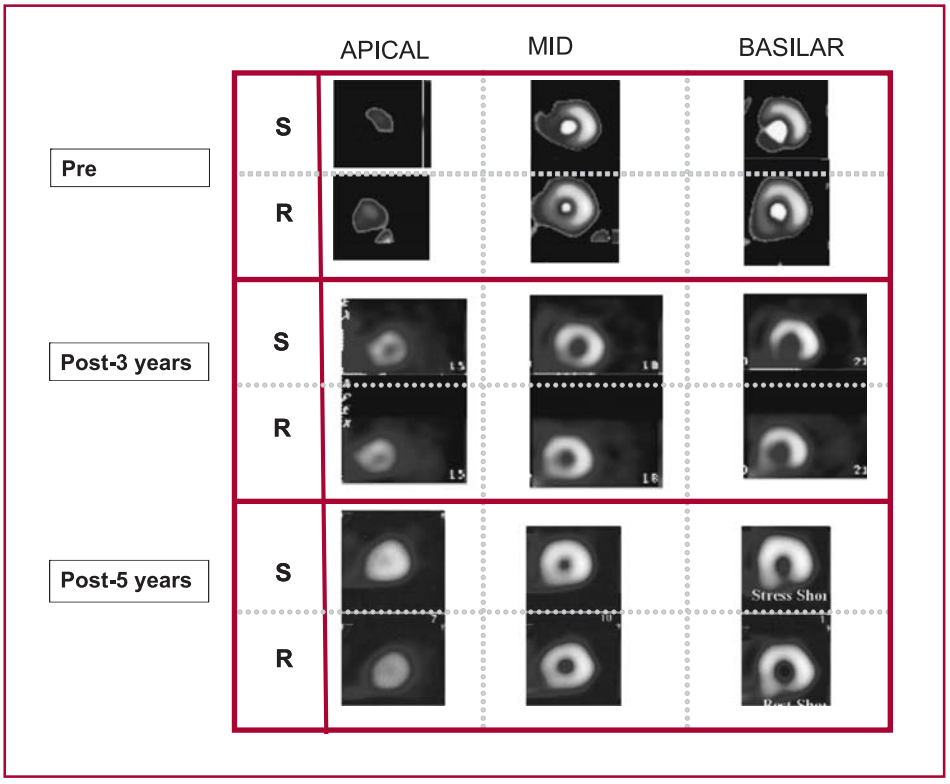

Fig. 2. Perfusion changes in one patient.

in the number of segments with nontransmural infarction and with ischemia. The number of segments with transmural compromise decreased from 15 to 3 (risk reduction of 80%; p=0.0005). Analysis of nontransmural segments should be performed in a comprehensive fashion. The global number of these segments increased from 7 to 9; however, preoperative nontransmural segments originally reported decreased from 7 to none. It might be considered that the global increase of these nontransmural segments corresponded to segments incorporated not only as a consequence of the progression of the disease but also at the expense of transmural segments, in a clear reduction of the fibrotic tissue. Although this series has a small number of patients to draw definite conclusions, it describes the outcomes of the first worldwide series of grafted patients 5 years after the procedure. We believe it is our ethical duty to inform the outcomes of the first investigations, provided they have been carried out with innovative techniques.

RESUMEN

Implante cardíaco de mioblastos. Resultado en el seguimiento a cinco años

Las primeras experiencias en la búsqueda de regeneración cardíaca en fase clínica se llevaron a cabo con mioblastos en diversos centros del mundo, entre los que se cuentan instituciones de la República Argentina. Esto motivó en su momento publicaciones en los años 2002 y 2003 sobre los estudios iniciales. Posteriormente se realizó una evaluación de estos pacientes a los tres años de seguimiento. Es nuestro interés presentar en el mismo grupo tratado un nuevo análisis superados los cinco años del implante.

Si tomamos en cuenta los segmentos comprometidos y los dividimos en infarto transmural, infarto no transmural, isquémicos y normales, hallamos que sobre 68 segmentos pasibles de estudio en los 4 enfermos sobrevivientes a los 66 ± 6,2 meses hubo un claro retroceso de los segmentos con infarto transmural y un incremento en los segmentos no transmurales e isquémicos. Los segmentos con compromiso transmural retrocedieron de 15 a 3, lo que representa una reducción del 80% (p = 0,0005). El análisis de los segmentos no transmurales debe ser exhaustivo. Si bien globalmente aumentaron de 7 a 9, los segmentos no transmurales registrados originariamente en el preoperatorio descendieron de 7 a ninguno. Se puede considerar que el aumento global de estos segmentos no transmurales correspondió a segmentos incorporados tanto por el avance de la enfermedad como a expensas de los transmurales, en claro retroceso del tejido fibrótico.

Palabras clave > Mioblastos - Trasplante de células - Insuficiencia cardíaca

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