



Clinical research

Autologous intramyocardial injection of cultured skeletal muscle-derived stem cells in patients with non-acute myocardial infarction

Jesús Herreros^a, Felipe Prósper^{b*}, Ana Perez^b, Juan J. Gavira^a, María José García-Velloso^c, Joaquín Barba^a, Pedro L. Sánchez^e, Consuelo Cañizo^d, Gregorio Rábago^a, Josep M. Martí-Climent^c, Milagros Hernández^b, Natalia López-Holgado^d, José María González-Santos^e, Cándido Martín-Luengo^e, Eduardo Alegria^a

^aDepartment of Cardiology and Cardiovascular Surgery, Clínica Universitaria, Universidad de Navarra, Pamplona, Spain

^bHematology and Cell Therapy Area, Clínica Universitaria, Universidad de Navarra, Pamplona, Spain

^cDepartment of Nuclear Medicine, Clínica Universitaria, Universidad de Navarra, Pamplona, Spain

^dDepartment of Hematology, Hospital Clínico Universitario de Salamanca, Salamanca, Spain

^eDepartment of Cardiology and Cardiac Surgery, Hospital Clínico Universitario de Salamanca, Salamanca, Spain

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KEYWORDS

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Aim Experimental animal studies suggest that the use of skeletal myoblast in patients with myocardial infarction may result in improved cardiac function. The aim of the study was to assess the feasibility and safety of this therapy in patients with myocardial infarction.

Methods and results Twelve patients with old myocardial infarction and ischaemic coronary artery disease underwent treatment with coronary artery bypass surgery and intramyocardial injection of autologous skeletal myoblasts obtained from a muscle biopsy of vastus lateralis and cultured with autologous serum for 3 weeks. Global and regional cardiac function was assessed by 2D and ABD echocardiogram. ¹⁸F-FDG and ¹³N-ammonia PET studies were used to determine perfusion and viability. Left ventricular ejection fraction (LVEF) improved from 35.5±2.3% before surgery to 53.5±4.98% at 3 months ($P=0.002$). Echocardiography revealed a marked improvement in regional contractility in those cardiac segments treated with skeletal myoblast (wall motion score index 2.64±0.13 at baseline vs 1.64±0.16 at 3 months $P=0.0001$). Quantitative ¹⁸F-FDG PET studies showed a significant ($P=0.012$) increased in cardiac viability in the infarct zone 3 months after surgery. No statistically significant differences were found in ¹³N-ammonia PET studies. Skeletal myoblast implant was not associated with an increase in adverse events. No cardiac arrhythmias were detected during early follow-up.

Conclusions In patients with old myocardial infarction, treatment with skeletal myoblast in conjunction with coronary artery bypass is safe and feasible and is associated with an increased global and regional left ventricular function,

* Correspondence to: Felipe Prósper, Hematology and Cell Therapy Area, Clínica Universitaria, Universidad de Navarra, Av Pio XII 36, Pamplona 31009, Spain. Tel: +34 948 255400; fax: +34 948 296500

E-mail address: fprosper@unav.es (F. Prósper).

improvement in the viability of cardiac tissue in the infarct area and no induction of arrhythmias.

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Introduction

Myocardial infarction (MI) is associated with loss and dysfunction of cardiomyocytes and leads to heart failure and death as a consequence of irreversible cell loss. Unlike other tissues, the heart muscle has a very limited if any, capacity of regeneration. Lack of stem cells in the heart and potential of the damaged heart cells to undergo repair or divide at least to a significant extent are the main reasons for this inability.^{1,2} Medical treatment or revascularization can improve the function of viable or hibernating myocardium, but cannot restore necrotic myocardial tissue.

Cellular transplantation has emerged as a potential alternative to heart transplant for patients with end-stage cardiac disease.^{3,4} With the aim of replacing necrotic tissue, cells from different sources have been implanted in animal models of myocardial infarction including embryonic stem cells,⁵ fetal cardiomyocytes,⁶ skeletal myoblast,⁷ haematopoietic stem cells,⁸ mesenchymal stem cells⁹ or endothelial progenitor cells.¹⁰ Most of these studies have invariably shown engraftment of donor cells and reconstitution of heart structures, i.e., cardiomyocytes and blood vessels, and most of them have been associated with improved heart function. Recently, the results from clinical trials of intramyocardial cell transplantation with bone marrow mononuclear cells (BMMNC)^{11,12} or bone marrow derived AC133 positive cells¹³ for cardiac regeneration have been reported.

Injection of skeletal myoblast in animal models of myocardial infarction and more recently in humans has been associated with successful engraftment of skeletal muscle fibers, graft survival and functional benefit.^{7,14–22} Although controversial, some evidences suggest that skeletal muscle progenitors may acquire certain characteristics of cardiac muscle both in vitro and in vivo.^{19,22–24}

We have examined, in a phase I clinical study, the feasibility and safety of intramyocardial transplantation of autologous skeletal myoblast cultured in vitro with autologous serum in patients with old myocardial infarction undergoing coronary artery bypass surgery. Our results show that the procedure is safe and feasible. Furthermore, functional as well as viability studies suggest that the combination of bypass surgery with implantation of autologous skeletal myoblast is associated with improvement in cardiac contractility and viability of the scarred tissue.

Patients and methods

Patient population and study design

A total of 12 patients were included in the study. Inclusion criteria were: (1) >4 weeks previous history of myocardial infarction

with an akinetic or dyskinetic non viable scar, as demonstrated by lack of metabolic activity on fluorine-18 fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography (PET); (2) indication for coronary artery bypass graft (CABG); (3) age 30–80; and (4) left ventricular ejection fraction greater than 25%. Exclusion criteria included (1) inability to obtain a myoblast cell culture; (2) positive serologic test for HIV or hepatitis; (3) previous history of malignant arrhythmias or muscular dystrophy; and (4) abnormal liver or kidney function test. The protocol and all the procedures were approved by the Institutional Review Board for Human Studies and Ethics Committee, the Regional Review Board for Clinical Trials with Human Subjects and the Spanish Health Authorities. All patients signed an informed consent before entering the study.

Baseline studies

Before surgery all patients underwent ¹³N-ammonia and ¹⁸F-FDG positron emission tomography scan, echocardiogram and 24 h Holter-ECG to determine function and viability of the heart muscle. Laboratory studies included cardiac and liver enzymes.

Echocardiographic studies

Global and regional myocardial contractility were measured by two dimensional echocardiography using a Sonos 5500 ultrasound system (Philips). Regional left ventricular wall motion analysis was performed as described by the committee on Standards of the American Society of Echocardiography,²⁵ dividing the left ventricle into 16 segments and scoring wall motion as 1=normal, 2=hypokinesis, 3=akinesis, 4=dyskinesis for each segment. The wall motion score index (WMSI) was calculated as the sum of the scores of the segments divided by the number of segments evaluated. WMSI was calculated for segments treated and non-treated with cell implant. Left ventricular ejection fraction (LVEF) was also calculated by using automatic border detection system (ABD).²⁶ Regional contractility was also assessed by colour-kinesis and tissue-Doppler for each segment. All studies were performed by two different observers blinded to clinical data. Reproducibility values within studies were 2.8±6.4 ml (CV 5.5%) for left ventricular end-diastolic volume and 0.3±4.6 (CV 6.6%) for LVEF.

Positron emission tomography studies

Myocardial blood flow and glucose metabolism were measured by PET scans before treatment. The perfusion and metabolism studies were performed with a whole-body positron emission tomograph (Siemens/CTI ECAT EXACT HR+, Knoxville, USA) which acquires 63 transaxial planes with an interplane spatial resolution of 4.5 mm. The ¹⁸F-FDG and ¹³N-ammonia were produced by in-site cyclotron (Cyclone 18/9, Ion Beam Applications, Belgium) and the radiopharmaceutical processing equipment.

Image acquisition: Before each study, a 2-min transmission scan was used to position the heart within the field of view, followed by a 5-min transaxial transmission scan using germanium-68 line sources to measure the photon attenuation correction. Intravenous injection of ¹³N-ammonia (9.25 MBq per kg, maximum 740 MBq) was slowly infused at a constant rate of 10 ml/min and acquisition was started at the beginning of the injection. Serial images were acquired for 20 min in a dynamic

sequence with varying frame duration (12×10 s, 4×15 s, 4×30 s, 3×300 s). This protocol has been previously described by Muzic et al.²⁷ After acquisition of the ¹³N-ammonia study, a period of 50 min was allowed for the physical decay of the ¹³N-ammonia radioactivity (physical half-life 9.9 min).

The glucose metabolic studies were performed using the hyperinsulinemic-euglycaemic clamp technique which allows to differentiate inflammation.²⁸ ¹⁸F-FDG was injected as an intravenous bolus after stabilization of the glucose level between 85 and 95 mg/dl (4.6 MBq per kg, maximum 370 MBq). The acquisition of the ¹⁸F-FDG images started at the injection time and serial images were recorded for 60 min (8×15 s, 2×30 s, 2×120 s, 1×180 s, 4×300 s, 3×600 s), according to Knuuti et al.²⁸

Image processing: The reconstructed transmission images were segmented prior to their utilization in the attenuation correction of the emission data sets. The frames of the metabolic study were reconstructed using ordered subsets expectation maximization (OSEM) with two iterations and eight subsets. A Gaussian smoothing filter of 6 mm FWHM was applied. The transaxial images were reoriented in left ventricular short-axis, vertical long axis and horizontal long axis views for visual analysis. Six contiguous short-axis cross sections of the mid left ventricle were used for quantitative analysis. Tracer uptake was calculated for different regions and a quantitative absolute numerical value were assigned Regional myocardial blood flow (MBF) was calculated according to a three-compartment model,²⁹ and Patlak graphical analysis was applied to estimate regional myocardial glucose utilization rates (rMGU) from ¹⁸F-FDG PET data glucose utilization.²⁸

Autologous skeletal myoblast cell culture

Three weeks before CABG surgery a muscle biopsy was obtained from the vastus lateralis under sterile conditions and local anaesthesia (2% lidocaine hydrochloride) and processed immediately to obtain muscle progenitor cells as described with modifications.⁷ Briefly, muscle biopsy was stripped of connective tissue, minced and digested with trypsin/EDTA (0.5 mg/ml trypsin and 0.53 mm EDTA, GIBCO-BRL) and collagenase (0.5 mg/ml, GIBCO-BRL) to release satellite cells. After filtering, cells were grown in 79% Ham-F12 media (GIBCO-BRL) supplemented with 20% autologous serum and 1% penicillin/streptomycin (GIBCO-BRL). All patients underwent a plasma exchange the day before muscle biopsy using heparine as anticoagulant. Coagulation proteins were eliminated by neutralization of heparin with protamine sulphate. After testing for bacterial contamination, aliquots of 50 ml were cryopreserved until usage. Cell cultures were incubated at 37 °C and 5% CO₂ and passage of the culture was performed at sub-confluence to prevent myotube formation. During the first passage, preplating was applied to eliminate contamination of myoblasts with fibroblasts. Myoblasts were harvested after 3–4 passages for implantation. Myoblast purity was measured by flow cytometry and staining with monoclonal antibodies against human N-CAM (CD56), CD45 and desmin. These culture conditions proved to be equivalent to the use of fetal calf serum in terms of muscle progenitor yield and purity (data not shown). Myoblast and muscle progenitor cells are CD56 positive, desmin positive, CD45 negative.^{17,30}

CABG surgery and cell implantation

Three to 4 weeks after muscle biopsy all patients underwent conventional aortocoronary bypass surgery. During cardiopulmonary bypass surgery, after all graft sutures had been finalized immediately before removing extracorporeal circulation and

while the heart was initiating spontaneous heartbeat, muscle progenitor cells (myoblast) were injected subepicardially by multiple injections with an angled needle (Steriseal Ophthalmic canula 23G, Maersk Medical Ltd. Redditch, B98 9NL GB) that allows tangential injection of cells under the epicardium. Myoblasts were implanted in those segments previously identified by echocardiography as akinetic or dyskinetic in and around the infarct. Areas receiving cells were identified before surgery by echocardiogram and these same areas were analyzed during follow-up to determine changes in regional contractility. Before proceeding to myoblast implantation a sample of the harvested myoblasts was used for microbiology cultures, including gram staining to determine culture contamination.

Follow-up procedures

After surgery and before discharge, patients were monitored with continuous telemetry. Cardiac enzymes were analysed every 6 h during patient hospitalization. Inflammation was monitored by measuring the serum levels of C-reactive protein. All patients received a dose of methylprednisolone of 500 mg after surgery to prevent inflammation of the myocardium and oral amiodarone for 3 months to prevent cardiac arrhythmias.

Outpatient follow-up included ¹³N-ammonia and ¹⁸F-FDG PET 3 months after surgery and echocardiogram 40 days and 3 months after surgery for assessment of cardiac function. The presence of arrhythmias was monitored by Holter-ECG 40 days and 3 months after CABG.

Statistical analysis

Statistical analysis was performed with the SPSS 10.0 for windows software package. Comparisons were performed using the paired t-test, Wilcoxon rank-sum test, ANOVA for repeated measurements or Friedman test depending on the Shapiro–Wilk and Kolmogorov–Smirnov normality test. Descriptive analysis is presented as mean (SEM) for quantitative variables or median (IQR) for categorical variables. Statistical significance was achieved if *P* values were less than 0.05.

Results

The demographic, clinical and functional characteristics of patients are included in Table 1. Mean follow-up for all patients was 6.5 months (1.27). Initial therapy for acute MI included fibrinolysis in seven patients, one primary angioplasty and one differed angioplasty. Three patients were managed with conservative treatment. Differences in therapy are explained by the differences in standard treatment according to the time when each patient was treated for the acute MI.

Bypass surgery and cell transplantation

Patients were treated with bypass surgery and skeletal myoblast transplant. Revascularization was based not on the existence of tissue with residual viability but rather on clinical data suggesting that moderate improvement in a low percentage of non viable segments can be obtained with revascularization. Revascularization involved both areas with infarct and areas with viable or hibernating tissue.

The median number of grafts was 2 (3). Revascularization to areas of cell implantation was performed in all

Table 1 Characteristics of the patients^a

Patient	Age	Sex	MI location	MI age (months)	Symptoms angina	NYHA baseline/ FU	Bypass location	Muscle biopsy	Myoblast implanted	LVEF baseline 2D/ ABD	LVEF FU 2D/ ABD
1	63	M	Anterior	4	Unstable	III/II	LAD, RC OM	10 g	318	35/37	55/56
2	64	M	Anterior-apical	6	III	II/I	LAD, OM	13 g	165	40/45	50/53
3	40	M	Inferior	5	Unstable	II/I	RC, LAD	7.5 g	192	45/46	70/65
4	55	M	Anterior	23	I	II/I	LAD, RCOM	7 g	393	40/47	62/66
5	68	M	Anterior-apical	168	III	III/II	LAD, RCOM	10 g	200	27/26	40/50
6	74	F	Anterior	10	Unstable	III/III	LAD	9 g	110	30/38	40/48
7	69	M	Inferior	125	Unstable	II/no FU	LAD, RC, OM, Dg	9.5 g	171	40/38	No FU
8	71	M	Inferior	108	Unstable	II/I	LAD, OM	5.5 g	105	40/42	47/51
9	56	M	Anterior	120	III	I/I	LAD, Dg OM	9 g	0	45/40	35/38
10	74	M	Inferior	20	III	II/no FU	LAD, OM, Dg, RC	14 g	390	25/29	No FU
11	68	M	Anterior	3	I	I/I	LAD, RC, OM	10 g	100	40/43	51/49
12	73	M	Anterior-apical	146	III	III/II	LAD, Dg, OM	11.3 g	180	43/41	46/45

^aAbbreviations: MI=myocardial infarction; NYHA=New York Heart Association; FU=follow-up assessment was considered 3 months after surgery; LAD=left anterior descending coronary artery; RC=right coronary artery; OM=obtuse marginal branch; Dg=diagonal coronary artery; Myoblast implant=number of cells $\times 10^6$; LVEF=left ventricular ejection fraction; 2D=bidimensional; ABD=automatic border detection.

but one patient. Those cardiac segments akinetic and dyskinetic received both revascularization and cell implant while revascularization was the only treatment for the other myocardial segments.

The mean size of the muscle biopsy was 9.45 g (0.81 g) and the mean volume of autologous serum obtained was 1.735 ml (327.5 ml). After a median of 23 (15) days in culture, a mean of 221×10^6 (100.37×10^6) myoblast were obtained with a purity of 65.6% (18.2%) (percentage of CD56 positive cells/CD45 negative cells). An average of 5 ml (3.5 to 6 ml) of solution containing skeletal myoblast was injected by multiple injections as described in methods. The average concentration of myoblast was 50×10^6 cells/ml and the number of injections varied between 5 and 15. Besides the 12 patients receiving skeletal myoblast, two further patients underwent a muscle biopsy but could not receive cells due to the inability of myoblasts to grow in culture. These patients were not included in the study as determined in the exclusion criteria.

Adverse events

Plasma exchange was not associated with adverse events which is consistent with the fact that at a given time the total extra vascular volume in the apheresis circuit was less than 170 ml. All patients were discharged from the hospital and are alive at the time of this report. The median number of hospitalization days was 9 (3) and the mean duration of extracorporeal circulation was 156 min (55 min). Myocardial inflammation due to the procedure was indirectly assessed by measuring the levels of C-reactive protein which were not significantly different from baseline at 40 days and 3 months follow-up (data not shown).

Patient 9 could not receive the myoblast implant because a positive gram staining was detected immediately before implantation. This patient underwent all protocol scheduled studies during the follow-up.

No ventricular tachyarrhythmias were detected after treatment with bypass surgery and skeletal myoblast implant during the hospitalization period or during out-patient follow-up. The median number of premature ventricular beats was 16 (393) and 6 (19) at baseline, post surgery and at 3 months follow-up respectively ($P=ns$). Placement of automatic internal cardioverter-defibrillator was not required in any of our patients. However, patient 6 developed a non-sustained ventricular tachycardia 40 days after surgery. In this patient aneurysmectomy with the Dor technique had also been performed.

Left ventricular function

Left ventricular ejection fraction (mean \pm SEM) was increased from $35.5 \pm 2.3\%$ before surgery to $53.5 \pm 4.98\%$ at 3 months ($P=0.002$) calculated by 2D echocardiography and from $39.8 \pm 3.26\%$ before surgery to $56.3 \pm 3.1\%$ ($P=0.001$) by ABD calculation (Fig. 1). The median number of akinetic/dyskinetic segments before surgery was 7 (2.25) and was reduced to a median of 3 (1.5) at

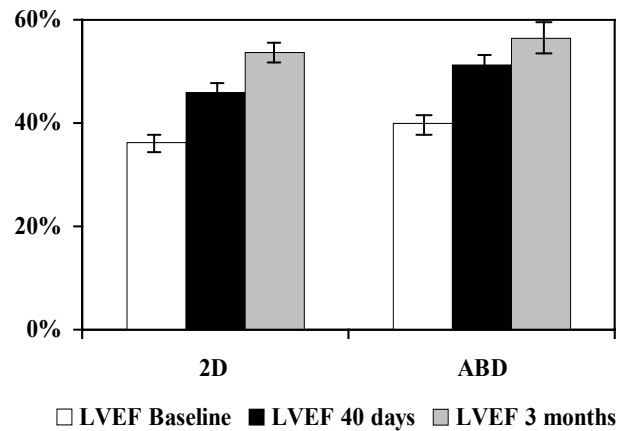


Fig. 1 Left ventricular function assessed by analysis of left ventricular ejection fraction (LVEF) calculated with 2D and ABD echocardiogram. Results represent mean \pm SEM. $P=0.002$ between LVEF baseline and 3 months (2D); $P=0.001$ between LVEF baseline and 3 months (ABD).

Table 2 Regional function (Wall motion score index)

	Baseline	40 days	3 months	P^a
Global	1.73 \pm 0.14	1.40 \pm 0.07	1.25 \pm 0.07	0.0001
Treated segments	2.64 \pm 0.13	2.03 \pm 0.16	1.64 \pm 0.16	0.0001
Untreated segments	1.29 \pm 0.13	1.1 \pm 0.06	1.05 \pm 0.04	0.02

^a P : comparison between WMSI baseline and at 3 months follow-up. Results represent the mean \pm SEM.

3 months after myoblast implant ($P=0.01$). The WMSI (mean and SEM) was 1.73 ± 0.14 at baseline versus 1.25 ± 0.07 at 3 months ($P=0.0001$) (Table 2). To try to better differentiate the potential benefit on cardiac function due to myoblast transplant and to revascularization, we examined the improvement in the WMSI in those segments in which myoblasts had been implanted along with revascularization and in those that did not receive cells but only revascularization. Although there was a significant reduction in the WMSI for segments treated and non-treated, these differences were greater in the case of treated areas (Tables 2 and 3). The median NYHA class was reduced at 3 month follow-up from 3 baseline to 2, 3 months post surgery ($P=0.007$).

Myocardial perfusion and viability studies

In seven out of 12 patients both ^{18}F -FDG and ^{13}N -ammonia PET were performed before surgery (1–5 days) and 3 months after surgery. Before surgery the mean glucose uptake value was $0.158 \pm 0.026 \mu\text{mol g}^{-1} \text{min}^{-1}$ for the whole myocardium and 3 months post surgery was $0.270 \pm 0.008 \mu\text{mol g}^{-1} \text{min}^{-1}$ ($P=0.012$). When we analysed the area of necrotic tissue due to myocardial infarction (infarct area) there was a significant increase in ^{18}F -FDG uptake suggesting increased myocardial viability in the infarct area (Table 4). Although an increase in ^{13}N -

Table 3 Regional function (Wall motion score index) in each patient for those segments treated with revascularization and cells and those treated with revascularization alone

	Segments treated/ non-treated	Baseline WMSI treated/ non-treated	3 months WMSI treated/ non-treated
UPN 1	4	3.1	1.75
	12	1.08	1
UPN 2	7	3.1	1.4
	9	1.81	1.18
UPN 3	4	3.2	1
	12	1.16	1
UPN 4	3	3	2
	13	1	1
UPN 5	5	3.3	2
	11	1.5	1.16
UPN 6	6	3.2	2.2
	10	1.22	1
UPN 7	4	3.1	1.8
	12	1.3	1
UPN 8	5	3.3	2
	11	1.6	1.16
UPN 9	—	—	—
UPN 10	—	—	—
UPN 11	5	3.4	2.4
	11	1.18	1
UPN 12	7	3.57	2.8
	9	1.12	1

ammonia uptake was detected after treatment with revascularization and myoblast implant, these differences were not statistically significant either for the whole myocardium or even when the analysis was restricted to the infarct area. In Fig. 2A we show the ^{18}F -FDG and ^{13}N -ammonia PET from a representative patient at baseline and at 3 months post surgery. In Fig. 2B we show the ^{18}F -FDG and ^{13}N -ammonia PET from patient number 9 who did not receive skeletal myoblast due to cell contamination but otherwise underwent the same surgical procedure and follow-up. Arrowheads indicate areas with lack of viability.

Discussion

The main findings of our study can be summarized as follows: (1) Transplantation of autologous skeletal myoblast directly into the myocardium in patients undergoing

CABG surgery and with a history of MI is safe and feasible. (2) Treatment with bypass surgery and myoblast injection in segments with abnormal WMSI results in both improvement in myocardial contractility (decreased WMSI) and increased in tissue viability. Differences in WMSI at baseline and 3 months after treatment are greater when we consider segments treated with revascularization and cell implant. (3) Increase uptake of glucose by ^{18}F -FDG PET is observed in the infarct area implanted with skeletal myoblast, 3 months post surgery.

The use of stem cells and in particular of skeletal myoblast for cardiac repair in patients with myocardial infarction has been an active field of research for the last few years. It has been demonstrated both in large and small animal models that these cells engraft, differentiate into skeletal fibers, acquire certain characteristics of cardiac muscle and contribute to improve cardiac function and animal survival.^{16,20,21,23} Very recent reports in patients with myocardial infarction have shown that skeletal autologous myoblast can survive after transplantation and form viable grafts in heavily scarred human myocardial tissue thus providing a proof of principle.^{17,22,31}

Although skeletal myoblast can be successfully transplanted, the percentage of cells that survive and differentiate into functional tissue is currently unknown and some reports suggest that it may be lower than 1%.^{31,32} The implication is that a very large number of cells may be required for this therapy to work.

The number of cells injected in our patients (200×10^6 cells were injected subepicardially) was significantly lower than cells injected in the study from Menasche et al.³³ and similar to the number of cells injected in the study by Pagani et al. in which engraftment of myoblast was demonstrated.³¹ Although, proof of engraftment was not obtained in our patients, based on these two studies probably enough cells were injected to obtain a successful engraftment. The increase in ^{18}F -FDG uptake in the area where cells had been implanted suggest that viable tissue was present in the area of the infarct where previously no viable tissue could be detected. In any case, we cannot directly demonstrate contribution to contractility of implanted cells.

Although no control patients were included in the study, contamination of skeletal myoblast in one patient gave us the opportunity to assessed the effect of CABG on ^{18}F FDG PET. Remarkably, no significant changes were

Table 4 ^{13}N -ammonia ($\text{ml g}^{-1} \text{min}^{-1}$) and ^{18}F -FDG ($\mu\text{mol g}^{-1} \text{min}^{-1}$) positron emission tomography scan (PET)^a

		Baseline	3 months	P
Global	^{18}F FDG	0.158±0.026	0.270±0.008	0.012
	^{13}N ammonia	0.47±0.05	0.5±0.03	0.309
Infarct	^{18}F FDG	0.126±0.022	0.231±0.011	0.010
	^{13}N ammonia	0.36±0.04	0.39±0.01	0.537
Non infarct	^{18}F FDG	0.170±0.029	0.284±0.0.013	0.014
	^{13}N ammonia	0.59±0.07	0.62±0.06	0.365

^aResults represent the mean±SEM.

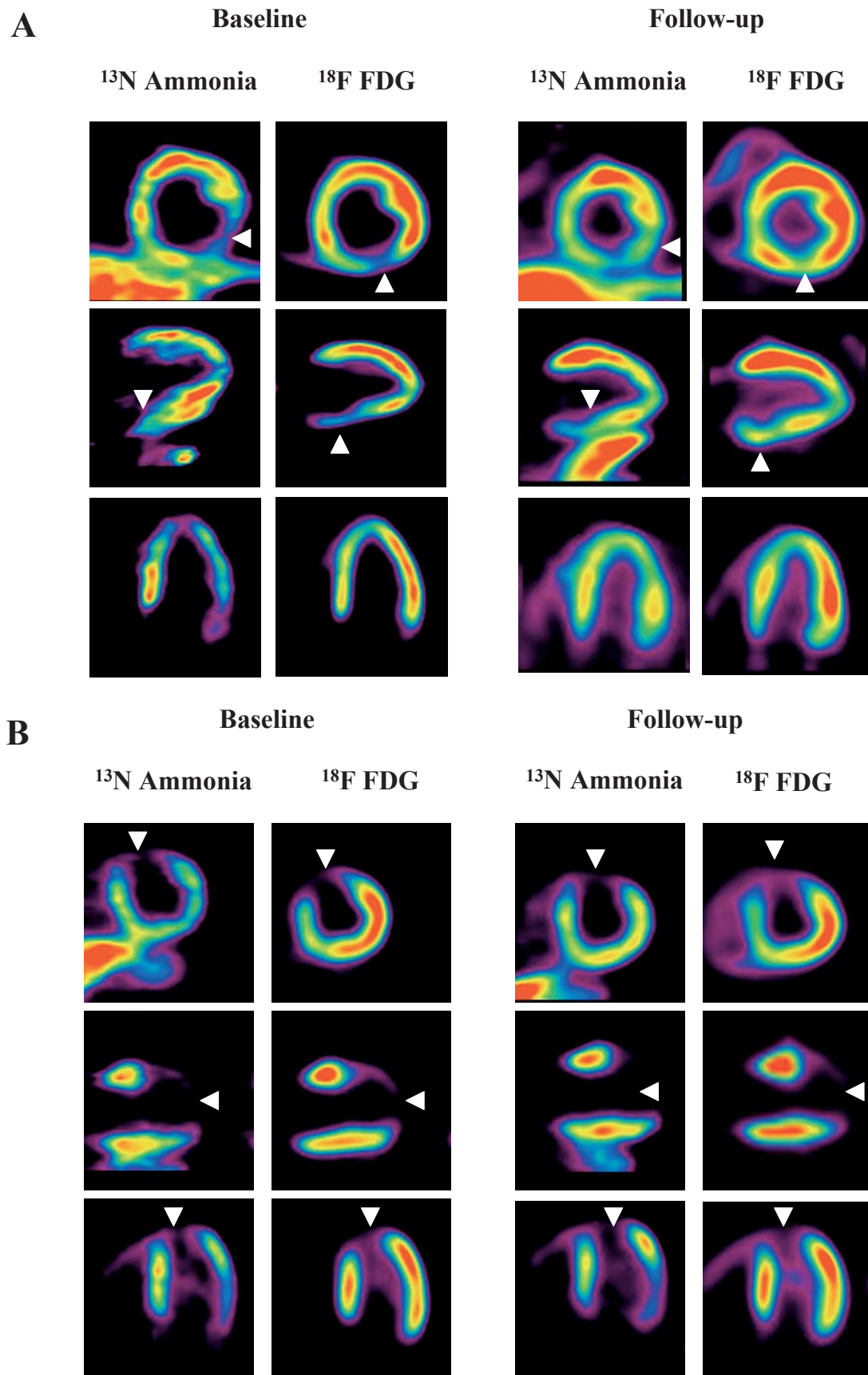


Fig. 2 ^{18}F -FDG and ^{13}N -ammonia PET. Baseline analysis and 3 months after surgery are represented. (A) Patient 5: a representative example of a patient with implanted myoblast. (B) Patient 9: no myoblast implant due to sample contamination. Arrows indicate infarct tissue before and after surgery.

observed 3 months after surgery in patient 9, not receiving skeletal myoblast (Fig. 2B). The results by no means demonstrate that the increase in glucose uptake is due to skeletal myoblast but is an interesting finding that supports the use of PET scan for monitoring viability of implanted cells in future studies.

Beside engraftment, proving that implanted cells contribute to cardiac function is of paramount relevance before this type of therapy can be applied clinically. Left ventricular cardiac function improvement has been well documented in animal models of MI^{7,21,34} despite the lack of understanding of the mechanism responsible for the effect.³⁵ Although some studies have suggested that myoblast acquire certain characteristics of cardiac muscle after transplantation into the myocardium (presence of cardiac specific MHC and connexin 43)^{18,21} and that at least in vitro, skeletal fibers and cardiac muscle can form electromechanical junctions³⁶ the overwhelming evidence suggest that this is not the case and that skeletal myoblast do not transdifferentiate into cardiomyocytes.^{24,37} The elastic properties of implanted fibers and/or the secretion of certain growth or survival factors by cells, contributing to remodelling or to recruitment of circulating or local cardiac stem cells have been implicated as potential mechanisms involved in the observed benefit of myoblast transplant in animal studies.³⁵

Unlike animal models, human studies have included patients subjected to bypass surgery and cell transplant preventing a clear conclusion as to the benefit derived from cell transplant. Our patients received both cells and revascularization to the same area, so the observed improvement in LVEF and WMSI can be attributed at least in part to revascularization. The fact that those segments of the heart treated with cells and revascularization experienced a greater improvement in WMSI indicates that it is unlikely that the only reason for cardiac improvement is the CABG surgery. Furthermore, it has been well demonstrated that re-perfusion of non viable and fibrotic tissue as a result of MI does not improve cardiac function or viability.³⁸

The lack of glucose uptake before surgery rules out that the tissue where the cells were implanted might have been hibernating tissue that could have become viable as a consequence of revascularization. In any case, to demonstrate that skeletal myoblast are responsible for improvement in cardiac function we will need to transplant the cells without any further therapy and currently this would not be ethical unless cells could be applied by percutaneous injection.

An interesting finding in our patients is the lack of cardiac arrhythmias observed, which is in contrast with recent studies^{31,33} in which four out of 10 patients with old MI treated with CABG and intramyocardial injection of skeletal myoblast required implantation of automatic internal cardioverter-defibrillator.³³ The cause of arrhythmias is at present unknown but it may be related to reentry electrical circuits due to the fact that skeletal myoblast do not established gap junctions with cardiomyocytes,²⁴ the number and/or volume of cells implanted or other currently unknown mechanisms. We

cannot rule out that the low incidence of arrhythmias in our patients is not due to the revascularization of the infarct area or even to patient selection as the baseline LVEF was better in our study than in either of the previous studies.^{31,33} In a very recent study, the finding that skeletal myoblast do not establish gap junctions with cardiomyocytes has been hypothesized as a mechanism to prevent the generation of deleterious extrasystoles.³⁷

Unlike other studies, our patients received myoblast cultured without fetal bovine serum. The use of xenogeneic proteins could be associated with an immune reaction and inflammation although only very limited signs of inflammation have been described in patients treated with skeletal myoblast.³¹ Whether the use of FCS serum could induce some inflammation that favours the incidence of arrhythmias is currently unknown. Similar results have been described by the group of Trainini et al. using autologous serum for in vitro expansion of skeletal myoblast in four patients undergoing CABG surgery and myoblast transplant (personal communication).

Other ways of transplanting stem cells as well as different sources of stem cells other than skeletal myoblast have been explored in patients.^{11–13,39} Results are challenging and cardiac function improvement have been suggested both in patients with acute and non acute myocardial infarction indicating that cardiac repair may be a reasonable goal within a few years, yet this field is just at its infancy.

In conclusion, our results suggest that cell transplantation with bypass surgery is associated with improvement in cardiac function, increased tissue viability and lack of side effects thus resulting in a promising therapy for patients with heart failure. We believed that these results warrant further clinical research including randomized studies.

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