

Intramyocardial injection of skeletal myoblasts: long-term follow-up with pressure–volume loops

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SUMMARY

The human heart has a limited capacity for self-repair because, unlike most other cells, cardiomyocytes do not regenerate. Therefore, if a substantial number of myocytes is lost after a myocardial infarction, the performance of the heart may become severely limited, leading to a condition of heart failure. Recently, cell transplantation has emerged as a potential therapy for patients with end-stage heart failure. Of the various cell types being investigated for this purpose, skeletal myoblasts are an attractive option, because they are readily available from muscle biopsies and, if autologous cells are used, immunosuppression is not required and ethical issues are avoided. Several studies have shown that the cells can survive and differentiate after transplantation, and promising clinical results have been reported. However, effects of this therapy on left ventricular function remain largely unknown. In the present study, we investigated the long-term hemodynamic effects of intramyocardial injection of autologous skeletal myoblasts in patients with ischemic heart failure. Our findings indicate hemodynamic improvement after follow-up for up to 1 year, which is especially promising in view of the expected decline in left ventricular function in these patients.

KEYWORDS cell transplantation, heart failure, left ventricular function, myoblasts, pressure–volume loops

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INTRODUCTION

Skeletal myoblasts harvested from a muscle biopsy are expanded *in vitro*, and after transplantation into the host, these mononucleated precursors proliferate and differentiate into contractile myotubes. Their high resistance to ischemia promotes their survival after injection into the target area, which is typically a post-infarct scar. These properties have been translated to successful animal studies, which showed that skeletal myoblasts can survive and differentiate in the host and replace infarcted myocardium.¹ Experimental studies have shown improvement in postinfarct cardiac function after skeletal myoblast transplantation.^{2–7} The proposed mechanisms of functional improvement include the involvement of grafted myoblasts in active contraction, alteration of elastic properties of the scar area, release of growth or angiogenic factors, and paracrine effects on resident cardiac precursor cells.⁸ Studies in rat models demonstrated that transplanted myoblasts retain excitable and contractile properties but that at least the large majority of these cells remain electrically and functionally isolated from neighboring cardiomyocytes.^{9,10} This suggests that active contraction might not be effective and might indicate that paracrine factors have a major role in the beneficial effects seen after myoblast grafting.⁶

Several clinical studies have now confirmed the animal data, showing cell survival and differentiation^{11,12} and improved global cardiac function after transplantation of autologous myoblasts.^{12–15} These studies have also raised the issue of potential arrhythmogenicity of this approach.¹⁶ In most ongoing trials, this potential problem is currently addressed by use of an implantable cardioverter-defibrillator and prophylactic administration of amiodarone. Hemodynamic follow-up of patients who received skeletal myoblast injections was based mainly on echocardiography, and uniformly improved left ventricular (LV) ejection fraction and improved regional contractility in the target area were reported.^{14,15,17} Smits *et al.*¹³, using

angiography, found an increased LV ejection fraction at 3-month follow-up, but nuclear radiography and magnetic resonance imaging failed to confirm this improvement. Thus, although echocardiographic follow-up indicates functional improvement, hemodynamic follow-up data are still limited and lack specificity.

We therefore studied systolic and diastolic LV function in patients with ischemic heart failure using invasive pressure–volume loop analysis. This approach provides load-independent indices of global and intrinsic systolic and diastolic LV function. These measurements were performed at baseline (before cell injection) and at 6- and 12-month follow-up.

METHODS

Patients

The study included five patients (mean age 60 ± 7 years; all men) with a New York Heart Association functional class¹⁸ \geq II (mean 2.4 ± 0.9) and a reduced LV ejection fraction (mean $31 \pm 7\%$, as determined by radionuclide angiography). All patients had a history of anterior myocardial wall infarction, and the wall thickness in the target area for myoblast injection was >5 mm (as determined by echocardiography). All patients were on a stable medication regimen (angiotensin-converting enzyme inhibitors, β -blockers, and statins) for heart failure. The study protocol was approved by the institutional review committee and all patients gave informed consent. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Muscle biopsy and cell preparation

Biopsy samples of the quadriceps muscle were taken and cells were processed and cultured as previously described.¹³ Briefly, a muscle biopsy of approximately 10 g was obtained under local anesthesia, placed in a preservation solution, and shipped under temperature-controlled conditions (2 – 8°C) to a specialized laboratory (BioWhittaker, Cambrex BioScience, Walkersville, MD, USA) for myoblast cell isolation and expansion. After a culture period of approximately 17 days, the harvested cells were shipped back to the hospital suspended in an injectable medium.

PROTOCOL

Baseline hemodynamic measurements

We obtained baseline hemodynamic data during right- and left-heart catheterization before cell transplantation. The catheterization procedure

included thermodilution cardiac output, left ventriculography, and coronary angiography. In addition, we acquired pressure–volume loops using a 7F combined pressure–conductance catheter (CD Leycom, Zoetermeer, the Netherlands), which was placed in the left ventricle via the femoral artery. Pressure–volume signals were displayed online and digitized at a sample frequency of 250 Hz (Leycom CFL; CD Leycom). LV volume was calibrated using thermodilution and hypertonic saline dilution, as previously described.^{19,20} After the calibration measurements, the thermodilution catheter was removed and replaced with a balloon-occlusion catheter (PTS sizing balloon; NMT Medical Inc., Boston, MA, USA), which was used to perform temporary preload reductions by occluding the vena cava. Pressure–volume loops were acquired in the steady state and during gradual preload reduction, which enabled the determination of systolic and diastolic pressure–volume relationships.²¹

Cell transplantation

Cell transplantation was performed in the cardiac catheterization laboratory immediately after the baseline hemodynamic measurements were taken. The injection procedure has been described in detail.¹³ Briefly, we used an 8F injection catheter (Myostar™, Cordis Corp., Warren, NJ, USA) to deliver 15 endocardial injections that covered the entire infarct area (except in patient 1, who received only 10 injections). Each injection, of 0.3 ml, contained approximately 10 million cells and, on average, patients received a total of 158 ± 71 million cells.

Follow-up hemodynamic measurements

At 6 and 12 months after the cell transplantation, the patients were re-evaluated in the catheterization laboratory using coronary angiography and left ventriculography, and all the hemodynamic measurements were repeated.

Data analysis

Analysis of the steady-state pressure–volume loops was performed using custom-made software. LV function was quantified by cardiac output, end-diastolic and end-systolic volume and pressure, LV ejection fraction, and maximal and minimal rate of LV pressure change (dP/dt_{MAX} , dP/dt_{MIN}). The time constant of relaxation (τ) was determined using phase-plot analysis. Stroke work was calculated as the area of the pressure–volume loop. LV systolic function was characterized by end-systolic elastance

Table 1 Characteristics and cell-transplantation data for five men given intramyocardial injections of autologous skeletal myoblasts after infarction of the anterior myocardial wall.

Patient number	Characteristics of patient				Cell transplantation ^a	
	Age (years)	NYHA class ¹⁸	MUGA ejection fraction (%)	Previous myocardial infarction	Number of injections	Number of cells injected (millions)
1	54	II	26	Anterior, 1999	10	240
2	64	II	31	Anterior, 1986 Inferior, 1999	15	50
3	54	II	36	Inferior, 1990 Anterior, 1994	15	200
4	58	II	35	Anterior, 1995	15	150
5	69	IV	21	Anterior, 1985 Inferior, 1991	15	150

^aAll cell-transplantation procedures were performed between January and May 2003. MUGA, radionuclide angiography using a multiple-gated acquisition scan; NYHA, New York Heart Association.

(E_{ES} ; the slope of the end-systolic pressure–volume relationship). The position of this relationship was quantified by its intercept at a fixed end-systolic pressure of 120 mmHg (ESV_{120}).²² In addition, we determined preload recruitable stroke work (the slope of the relationship between stroke work and end-diastolic volume), and the slope of the relationship between dP/dt_{MAX} and end-diastolic volume as load-independent indices of systolic function.^{23,24} The diastolic chamber stiffness (E_{ED}) was determined as the slope of the end-diastolic pressure–volume relationship.

Statistical analysis

We used a multiple linear regression of repeated measures analysis of variance to compare hemodynamic indices at 6- and 12-month follow-up with baseline. All data are presented as mean \pm SD. Statistical significance was considered at $P < 0.05$.

RESULTS

Clinical assessment

Baseline patient characteristics and cell-injection data are summarized in Table 1. For all patients, the skeletal muscle biopsy, cell-injection procedure, and hemodynamic measurements at baseline and at 6- and 12-month follow-up were uneventful. No serious adverse events were observed and hospitalization was not required in the follow-up period. In two patients, a single episode of nonsustained ventricular tachycardia was found on the Holter recordings: 1 week after cell transplantation in patient 5, and at 6-month follow-up in patient 4. None of the patients received an implantable cardioverter-defibrillator. Mean New York Heart Association functional

class was 2.4 ± 0.9 at baseline, 1.8 ± 0.4 at 6-month follow-up, and 2.2 ± 0.4 at 12-month follow-up; there were no significant changes relative to baseline values.

Left ventricular function

Steady-state pressure–volume loops and end-systolic and end-diastolic pressure–volume relationships at baseline and 6- and 12-month follow-up for all individual patients are shown in Figure 1. The corresponding hemodynamic data are summarized in Table 2. The volumetric data show a significantly increased cardiac output at 6- and 12-month follow-up. This increase in cardiac output is achieved by a reduction in end-systolic volume and a slight increase in heart rate (although these effects did not reach statistical significance), whereas end-diastolic volume remained unchanged. This suggests an improvement in systolic function, which is in line with the increase in ejection fraction (which was significant at 6-month follow-up), dP/dt_{MAX} (which was significant at 12-month follow-up), and a tendency for improved stroke work at 6- and 12-month follow-up. The load-independent slope of the pressure–volume relationship showed a positive trend, but only preload recruitable stroke work reached statistical significance at 12 months. The position of the end-systolic pressure–volume relationship, quantified by ESV_{120} , showed a significant reduction at both time points ($P = 0.023$ and $P = 0.053$, respectively), indicating improved systolic function. Diastolic function indices were not significantly altered at either 6- or 12-month follow-up.

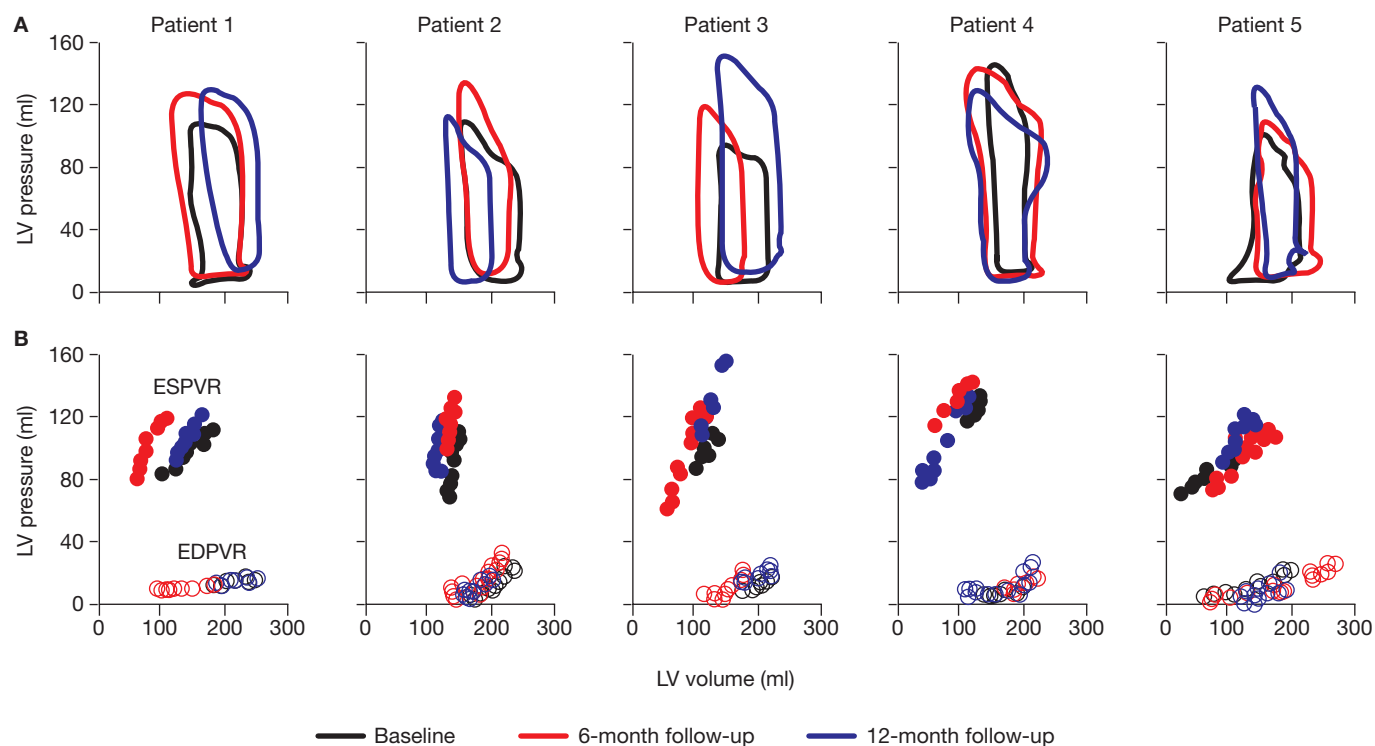


Figure 1 Steady-state pressure–volume loops (A), end-systolic pressure–volume relationship (ESPVR) and end-diastolic pressure–volume relationship (EDPVR) (B), for each patient. LV, left ventricular.

DISCUSSION

Autologous skeletal myoblasts have been advocated as a source for cell transplantation to treat patients with ischemic heart failure.^{25,26} The aim of the approach is to repopulate the necrotic tissue with living cells by injecting myoblasts directly into an infarcted area, thereby improving cardiac function or halting further decline. Compared with other candidate cell types, autologous skeletal myoblasts are advantageous because of their biological properties and availability, as well as an absence of immunologic and ethical concerns regarding their use.²⁷ A substantial number of experimental studies have documented the survival, proliferation, and differentiation of skeletal myoblasts into multinucleated myofibers after intramyocardial injection.^{1,11,12,28} Recent studies have confirmed these findings in human hearts.^{12,29} Animal studies have also provided evidence of improved cardiac function after cell transplantation in various models of ischemic heart failure.^{3,5,7}

However, the underlying mechanisms that bring about functional improvement remain unclear. Because the donor cells appear not to be functionally coupled with the host myocardium, a direct contractile contribution is unlikely.⁹

Furthermore, cell delivery by direct injection into the myocardium can invoke an injury that induces release of, for example, cytokines or promotes angiogenesis independent of the injected cells. Consequently, several alternative mechanisms have been proposed, including alteration of the elastic properties of the scar area, release of growth or angiogenic factors, and paracrine effects on resident cardiac precursor cells.⁶ These issues have been discussed extensively in several recent reviews.^{30,31}

The clinical experience with skeletal myoblast injection is still limited.^{12–15,17,32} The few reported studies have all been phase I trials, with few patients. These studies have shown the feasibility of this approach but have also raised concerns about potential arrhythmogenic effects.¹⁶ In all of the studies except that by Smits *et al.*,¹³ cell transplantation was combined with coronary artery bypass grafting or LV assist device implantation. Consequently, although these studies uniformly reported improved LV ejection fraction and contractility in the target area, information about the hemodynamic consequences of skeletal myoblast injection per se is virtually lacking. Furthermore, functional data were reported

Table 2 Hemodynamic indices in five men with a history of infarction of the anterior myocardial wall given intramyocardial injections of autologous skeletal myoblasts.

Index	At baseline	At 6-month follow-up	At 12-month follow-up	P values at follow-up vs baseline	
				6-month	12-month
HR (beats/min)	64 ± 10	67 ± 9	69 ± 11	0.397	0.091
Volumetric indices					
CO (l/min)	4.6 ± 0.9	5.6 ± 1.6 ^a	5.4 ± 1.5 ^a	0.016	0.036
ESV (ml)	175 ± 70	155 ± 81	164 ± 72	0.067	0.276
EDV (ml)	250 ± 65	247 ± 81	250 ± 64	0.794	0.982
Systolic function					
ESP (mmHg)	108 ± 19	120 ± 12	126 ± 14	0.229	0.097
EF (%)	33 ± 7	41 ± 11 ^a	37 ± 10	0.037	0.246
dP/dt _{MAX} (mmHg/s)	1,025 ± 236	1,130 ± 294	1,281 ± 328 ^a	0.317	0.031
SW (ml·mmHg)	6,511 ± 505	8,459 ± 2060	8,010 ± 2334	0.107	0.201
E _{ES} (mmHg/ml)	0.88 ± 0.82	0.81 ± 0.37	1.12 ± 0.67	0.738	0.288
ESV ₁₂₀ (ml)	190 ± 95	150 ± 107 ^a	157 ± 75	0.023	0.053
S-dP (mmHg/s per ml)	3.1 ± 1.6	3.9 ± 3.1	4.9 ± 1.8	0.499	0.146
PRSW (mmHg)	54 ± 12	56 ± 26	82 ± 13 ^a	0.843	0.050
Diastolic function					
EDP (mmHg)	18 ± 5	20 ± 6	21 ± 5	0.628	0.384
dP/dt _{MIN} (mmHg/s)	1,037 ± 227	1,124 ± 267	1,212 ± 215	0.400	0.111
τ (ms)	68 ± 12	71 ± 15	74 ± 13	0.445	0.155
E _{ED} (mmHg/ml)	0.15 ± 0.11	0.17 ± 0.11	0.19 ± 0.09	0.621	0.280

^aP < 0.05 versus baseline. CO, cardiac output; dP/dt_{MAX}, maximal/minimal rate of left ventricular pressure decrease, respectively; E_{ED}, end-diastolic stiffness; E_{ES}, end-systolic elastance; EDP, end-diastolic pressure; EDV, end-diastolic volume; EF, ejection fraction; ESP, end-systolic pressure; ESV, end-systolic volume; ESV₁₂₀, ESV at 120 mmHg; HR, heart rate; PRSW, preload recruitable stroke work; S-dP, slope of dP/dt_{MAX} versus EDV relationship; SW, stroke work; τ, relaxation time constant.

only in terms of LV ejection fraction after a short follow-up period.

Our study, therefore, is the first to investigate the effects of skeletal myoblast injection on systolic and diastolic LV function in patients. Our data indicate improved pump function (cardiac output) at 12-month follow-up, which appears to be mainly related to improved systolic function. Although there was a trend towards improvement in most systolic indices, statistical significance at 6-month follow-up was reached only for LV ejection fraction ($P=0.037$) and for the volume intercept of the end-systolic pressure–volume relationship (ESV₁₂₀, $P=0.023$), and at 12-month follow-up, dP/dt_{MAX}, ESV₁₂₀, and preload recruitable stroke work reached statistical significance ($P=0.031$, 0.053 , and 0.050 , respectively). Diastolic function remained unchanged, although inspection of the values might suggest

a slight decrease in diastolic function because of the gradual increase in end-diastolic pressure, τ, and diastolic stiffness.

Our findings relating to the LV ejection fraction are in line with those from previous clinical studies. The other indices can be compared only with available data from animal studies. Jain *et al.*³ studied pressure–volume relationships in hearts obtained from rats 4 weeks after myocardial infarction. The group that had received cell therapy (direct injection of skeletal myoblasts 1 week after myocardial infarction) showed an unchanged end-systolic pressure–volume relationship in comparison with control animals, whereas in the untreated animals (injection of vehicle) the end-systolic pressure–volume relationship was shifted to larger volumes. The slope of the end-systolic pressure–volume relationship was unchanged. Agbulut *et al.*⁷ compared injection of human skeletal myoblasts with injection of bone-marrow-derived

CD133⁺ progenitors in a nude rat myocardial infarction model. LV function was assessed 1 month after cell transplantation using pressure–volume loops. Compared with untreated control animals, the LV ejection fraction was higher in the myoblast-treated rats. Similarly, end-systolic elastance and preload recruitable stroke work were higher in myoblast-treated rats, but these differences were not statistically significant.

Thompson *et al.*⁵ performed a similar study in rabbits, using invasive LV pressure measurements and epicardial sonomicrometers to assess global and regional LV function. Furthermore, they performed measurements at baseline (i.e. after myocardial infarction, but before cell therapy) and after 4 weeks. Their results showed improved regional stroke work and systolic segment shortening in the myoblast-treated animals, but there was no change in LV end-diastolic pressure or dP/dt_{MAX} . Basically, those animal data are fully consistent with our findings in patients at 6-month follow-up. Unfortunately, only a limited number of indices were reported and thus the information on systolic and diastolic function is incomplete.

Furthermore, comparing groups of treated and untreated animals, as Jain *et al.*³ and Agbulut *et al.*⁷ did, is not the same as comparing measurements before and after treatment in individual patients, as in our study. In the former type of comparison, differences might result from the ability of the transplanted cells to slow down or halt the process of functional deterioration after myocardial infarction, whereas in the latter, differences reflect an actual improvement in LV function.

Several experimental studies have attributed the beneficial effects of cell transplantation mainly to a scaffolding effect, which would limit scar expansion.³³ However, in our study, all patients had a myocardial infarction at least 2 years before cell transplantation. Thus, myoblast-induced limitation of scar expansion is unlikely, which is supporting evidence for a true increase in contractility.

CONCLUSION

Although our study is obviously limited by its small sample size, the results are promising in showing improved pump function with a tendency for improved systolic function and unchanged diastolic function. These results were obtained with a follow-up period of up to 12 months, which, in view of the expected decline in LV function, indicates that injection of skeletal myoblasts has the potential to be an effective therapy in patients with ischemic heart failure.

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Competing interests

The authors declared they have no competing interests.

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