Cardiac bone marrow cell injection for chronic ischemic heart disease

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Chapter 10

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Intramyocardial Injection of Autologous Bone Marrow Mononuclear Cells in Patients with Chronic Myocardial Infarction and Severe Left Ventricular Dysfunction

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Abstract

Introduction: The present study investigated the safety, feasibility and potential efficacy of autologous bone marrow cell injection in patients with chronic myocardial infarction and severe left ventricular (LV) dysfunction.

Methods: In 15 patients (63±9 years, 14 men) bone marrow was aspirated from the iliac crest. Using the NOGA system (Biosense-Webster, Waterloo, Belgium), $94\pm14x10^6$ bone marrow-derived mononuclear cells were injected in the infarction border zone.

Results: Bone marrow cell injection was performed without periprocedural complications in all patients. At 2.5 months, 1 patient died from worsening heart failure. New York Heart Association class improved from 3.5±0.5 at baseline to 2.7±0.8 at 3 months (P<0.01), and 2.9±0.8 at 6 months (P<0.01 vs. baseline). LV ejection fraction (Tc-99m tetrofosmin gated single photon emission computed tomography) increased from 23±8% to 27±9% at 3 months (P=0.02) and regional wall thickening improved from 12.8±5.9% to 15.3±7.2% at 3 months (P=0.02). Injected myocardial segments displayed a significant improvement in regional wall thickening (6.6±6.3% vs. 11.7±7.0% at 3 months; P<0.01) and perfusion score (3.5±0.7 vs. 3.0±0.9 at 3 months; P=0.02), and a trend toward an improved F18-FDG score (2.9±0.9 vs. 2.6±1.0 at 3 months; P=0.06). Regional wall thickening (16.5±8.9% vs. 19.1±9.1% at 3 months; P=NS), perfusion score (1.8±0.4 vs. 1.7±0.5 at 3 months; P=NS) and F18-FDG score (1.7±0.4 vs. 1.6±0.4 at 3 months; P=NS) did not improve in non-injected myocardial segments.

Conclusion: Bone marrow cell injection in patients with chronic myocardial infarction and severe LV dysfunction is safe, feasible and appears to be associated with a decrease in heart failure symptoms and an improved LV function.

Introduction

The primary objective of the present study was to evaluate the safety and feasibility of intramyocardial bone marrow cell injection in patients with chronic myocardial infarction and severe left ventricular (LV) dysfunction. The secondary objective was to assess the effect of bone marrow cell injection on LV function, myocardial perfusion and glucose metabolism with the use of nuclear imaging.

Methods

Patients

From February 2005 to April 2006, 15 patients were recruited into the study. Patients were eligible for inclusion if they had severe heart failure symptoms (New York Heart Association (NYHA) class III or IV) despite optimized medical therapy, a history of myocardial infarction (documented by typical symptoms, increased cardiac enzymes, and typical electrocardiographic changes) at least 12 months before enrollment, a fixed perfusion defect as demonstrated by stress-rest Tc-99m tetrofosmin single photon emission computed tomography (SPECT), and a LV ejection fraction <40% as assessed by Tc-99m tetrofosmin gated SPECT.

Exclusion criteria for bone marrow cell injection were: 1. eligibility for percutaneous coronary intervention, coronary artery bypass grafting, valve surgery, surgical remodeling of the LV or cardiac resynchronization therapy, 2. a history of malignancy, 3. renal dysfunction (serum creatinine >200 µmol/L) and 4. unexplained hematological or biochemical laboratory abnormalities. The local medical ethics committee approved the protocol, and all patients gave written informed consent.

Study Protocol

Within 2 weeks before cell injection, a bicycle exercise test (to evaluate exercise capacity), Tc-99m tetrofosmin gated SPECT (to assess LV function and myocardial perfusion), and F18-FDG SPECT (to assess myocardial glucose metabolism) were performed. At hospital admission for cell injection, an independent physician who was blinded to all other clinical data scored the NYHA functional class and assessed quality-of-life with the use of the Minnesota Living with Heart Failure questionnaire.[1] This questionnaire contains 21 questions concerning a patient's perception of the effects of heart failure on daily life activities. Questions are scored from 0 to 5, resulting in a total score from 0 to 105, with the highest score reflecting the worst quality-of-life.

At 3 and 6 months, NYHA functional class, quality-of-life, exercise capacity and ventricular arrhythmia (24-hour Holter monitoring and device interrogation in patients with an implantable cardioverter-defibrillator (ICD)) were assessed. Tc-99m

tetrofosmin gated SPECT and F18-FDG SPECT were repeated at 3 months to evaluate LV function, myocardial perfusion and myocardial glucose metabolism.

Bone Marrow Aspiration, Cell Isolation and Cell Injection

On the morning of the injection procedure, bone marrow was aspirated from the iliac crest under local anesthesia. During mononuclear cell isolation (Ficoll density gradient), patients underwent non-fluoroscopic LV electromechanical mapping with the NOGA system (NOGAStar catheter, Biosense-Webster, Waterloo, Belgium). After completion of LV electromechanical mapping, the mapping catheter was replaced by a NOGA-compatible injection catheter (MyoStar catheter, Biosense-Webster). The injection catheter was prepared as previously described.[2]

Areas with unipolar voltage <6.9 mV were considered as the infarcted area[3] if these areas were geographically concordant with the perfusion defect on Tc-99m tetrofosmin SPECT. Autologous bone marrow-derived mononuclear cell injections were targeted at viable myocardium (unipolar voltage ≥6.9mV) located at the infarction border zone (**Figure 1**). Before every injection of cells into the myocardium, the catheter had to be positioned perpendicular to the endocardium with an excellent loop stability (<4 mm) and extension of the needle had to induce a premature ventricular contraction. Subsequently, 8 to 12 intramyocardial injections of approximately 0.2 ml each were delivered in the infarction border zone. The location of the injections were marked on the NOGA map.

Immediately after the injection procedure, continuous heart rhythm monitoring was started for 2 days. Laboratory markers of myocardial necrosis (creatinine kinase, troponin T) were collected 1 hour and 6 and 24 hours after cell injection. All patients continued their routine medical therapy after bone marrow cell injection. Before discharge, 2D echocardiography was performed to exclude postprocedural pericardial effusion.

Exercise Testing

All patients were subjected to a symptom-limited bicycle exercise test with a 20 Watt starting load and 10 Watt increment per minute before cell injection, at baseline, and at 3 and at 6 months follow-up. During the test, monitoring by a 12-lead electrocardiogram was carried out. Exercise capacity was assessed on the basis of the maximal load level achieved and expressed in Watts.

Tc-99m Tetrofosmin SPECT

Electrocardiographic-gated images at rest were obtained using 500 MBq Tc-99m tetrofosmin (radiation exposure 3.9 mSv). Imaging was performed as previously described with a triple-head SPECT camera system (GCA 9300/HG, Toshiba Corp., Tokyo, Japan).[4] Data were displayed in polar map format (normalized to the maximum tracer activity) and analyzed using a 17-segment model.[5] Two experienced observers blinded to all clinical data reviewed the images.

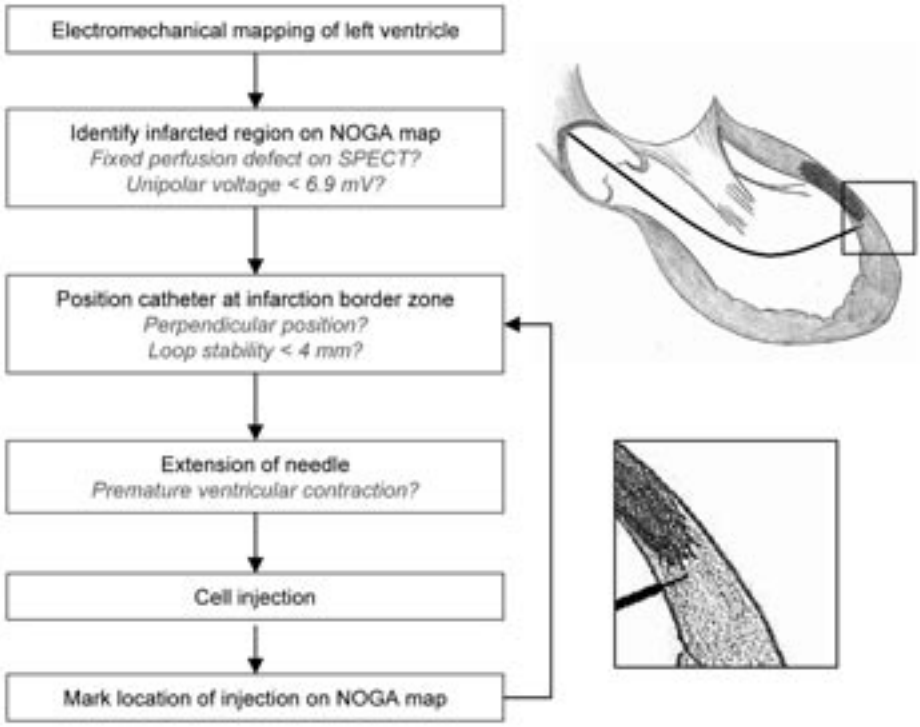

Figure 1
Flow chart providing an overview of how the cell injections were performed (left). Diagram illustrating how the injection catheter is advanced into the left ventricle through the aortic valve (right, upper panel). At the infarction border zone, the catheter tip is placed against the endocardial surface with the needle extended into the myocardium (right, lower panel).

Quantitative assessment of LV ejection fraction, LV end-diastolic and LV end-systolic volumes, and regional wall thickening was performed using previously validated and automated software (quantitative gated SPECT, QGS, Cedars-Sinai Medical Center, Los Angeles, California, USA). For each myocardial segment, regional wall thickening was determined as percentage increase in myocardial thickness from end-diastole to end-systole.[6] The intra- and inter-observer reproducibility for LV ejection fraction (r=1.00, r=0.98), LV end-systolic volume (r=1.00, r=0.98) and LV end-diastolic volume (r=1.00, r=0.99) were previously reported.[4]

Myocardial perfusion was analyzed quantitatively (17-segment model) with the use of QGS-software (Cedars-Sinai Medical Center). For each myocardial segment, segmental tracer activity was categorized on a 4-point scale: 1= tracer activity ≥75%, 2= tracer activity 50-75%, 3= tracer activity 25-50%, 4= tracer activity <25%. For each patient, segmental perfusion scores were summed and divided by 17 to yield the patients' segmental perfusion score, with the higher scores reflecting the worst perfusion. Intra- and interobserver agreements of 98% and 95% for analysis of segmental perfusion score were reported previously.[4]

F18-FDG SPECT

F18-FDG imaging, to evaluate myocardial glucose metabolism, was performed on a separate day, after Acipimox (Byk, The Netherlands) administration.[7] Acipimox increases myocardial F18-FDG uptake by decreasing the plasma level of free fatty acids.[8] After Acipimox administration, patients received a low-fat, carbohydrate-rich meal. This small meal further increases myocardial F18-FDG uptake by stimulating endogenous insulin release.[9] One hour after Acipimox administration, a blood sample was obtained to assess plasma glucose levels. When plasma glucose levels were between 5 and 7 mmol/L, 185 MBq F18-FDG (radiation exposure 3.5 mSv) was injected at rest. Forty-five minutes thereafter, data acquisition was started. Metabolic imaging was performed using the same SPECT system as described for perfusion imaging. F18-FDG short-axis slides were displayed in polar map format and normalized to the maximum activity (set at 100%). Polar maps were divided into 17 segments.[5] Two experienced observers blinded to all clinical data reviewed the F18-FDG images.

Tracer activity was analyzed quantitatively (in each myocardial segment) and categorized on a 4-point scale: 1= tracer activity ≥75%, 2= tracer activity 50-75%, 3= tracer activity 25-50%, 4= tracer activity <25%. For each patient, the segmental F18-FDG scores were summed and divided by 17 to yield the patients' segmental F18-FDG score, with the higher scores reflecting the worst perfusion. Intra- and interobserver agreements of 94% and 97% for the analysis of segmental F18-FDG score were reported previously.[4]

Statistical Analysis

Data were expressed as mean±SD. Comparison of continuous data was performed using the (un-)paired Student's t-test when appropriate. Categorical data were compared using Chi-square analysis. For all tests, a P-value <0.05 was considered statistically significant.

Results

The study population consisted of 15 patients (mean age 63±9 years, 14 men) with chronic myocardial infarction and severe LV dysfunction (**Table 1**). Patients had a history of myocardial infarction at least 12 months before enrolment and a fixed perfusion defect on Tc-99m tetrofosmin SPECT. All patients had severe heart failure symptoms despite optimized (if tolerated) medical therapy, including diuretics and oral anticoagulants in all, angiotensin-converting enzyme inhibitors in 93%, beta-blockers in 93% and spironolactone in 67%. Medication remained unchanged in the 3 months before cell injection and during the 6 months follow-up period.

Procedural Data

Total procedural time for mapping and cell injection was 53±14 min. Electromechanical maps comprised an average of 112±17 points. Patients received 10.1±1.1 injections of 0.2

Table 1. Clinical characteristics of the study population (n=15)

Characteristic		
Age (years)	63±9	
Men	14	(93 %)
Number of major coronary arteries narrowed ≥50%		
1	3	(20 %)
2	2	(13 %)
3	10	(67 %)
Time since myocardial infarction (years)	9±7	
Prior percutaneous coronary intervention	10	(67 %)
Prior coronary artery bypass grafting	9	(60 %)
Prior implantable cardioverter defibrillator	11	(73 %)
Hypertension	10	(67 %)
Diabetes mellitus	7	(47 %)
Hyperlipidemia (total cholesterol > 5 mmol/L)	13	(87 %)

ml in the infarction border zone. In total, the injected cell suspension contained 94±14x10^6 bone marrow-derived mononuclear cells. Cell viability was 98±1% and the CD34+ cell fraction was 2.6±1.3%.

Safety Data

Intramyocardial bone marrow cell injection was performed without periprocedural complications in all patients. Continuous electrocardiographic monitoring did not show sustained ventricular tachycardia and 2D echocardiography excluded postprocedural pericardial effusion. Laboratory values did not show evidence of infarction: preprocedural creatinine kinase was similar to in-hospital peak creatinine kinase (90±40 U/L vs. 101±54 U/L; P=NS) and in-hospital peak troponin T ranged between 0.03 µg/L and 0.18 µg/L (cut-off value for infarction >0.8 µg/L). All patients were discharged 2 days after the injection procedure.

One patient died 2.5 months after bone marrow cell injection as a result of worsening heart failure. During the 6 months follow-up period, 2 patients were hospitalized for decompensated heart failure at 3 and 5 months, respectively. Of note, in the 6 months before bone marrow cell injection 5 patients had been hospitalized for decompensated heart failure. Device interrogation in patients with an ICD and Holter registrations at 3 and 6 months revealed no sustained ventricular tachycardia.

Clinical Follow-up

Because 1 patient died at 2.5 months, repeat clinical evaluations were available in 14 patients. Assessment of patients' clinical status demonstrated a significant improvement in NYHA class from 3.5±0.5 at baseline to 2.7±0.8 at 3 months (P<0.01), and 2.9±0.8 at 6 months (P<0.01 vs. baseline; P=NS vs. 3 months). The individual data are presented in **Figure 2**.

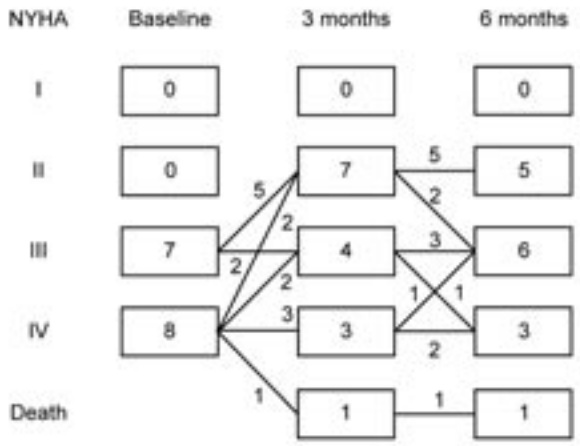

Figure 2
Individual New York Heart Association (NYHA) scores at baseline, at 3 and at 6 months follow-up. Mean NYHA class improved from 3.5±0.5 at baseline to 2.7±0.8 at 3 months (P<0.01), and 2.9±0.8 at 6 months (P<0.01 vs. baseline; P=NS vs. 3 months).

The mean number of days that patients were hospitalized for decompensated heart failure decreased from 3.4±5.8 in the 6 months before bone marrow cell injection to 0.6±1.6 in the 6 months after bone marrow cell injection (P=0.03).
The quality-of-life score improved from 51±10 to 38±19 at 3 months (P=0.02) and remained unchanged at 6 months (39±23; P=0.04 vs. baseline; P=NS vs. 3 months). Exercise capacity improved because the maximum level of stress achieved increased from 85±41 Watt to 100±41 Watt at 3 months (P=0.03) and 98±43 Watt at 6 months (P=0.04 vs. baseline; P=NS vs. 3 months).

Left Ventricular Function
As shown in **Figure 3A**, gated SPECT demonstrated a modest increase in LV ejection fraction from 23±8% at baseline to 27±9% at 3 months (P=0.02). Of interest, LV ejection fraction improved ≥5% in 7 of 14 patients. The increase in LV ejection fraction was mainly related a reduction in LV end-systolic volume (230±114 ml vs. 219±122 ml at 3 months; P=0.04), whereas LV end-diastolic volume remained unchanged (291±122 ml vs. 288±134 ml at 3 months; P=NS). In accordance with the improvement in global LV contractility, regional wall thickening increased from 12.8±5.9% to 15.3±7.2% at 3 months (P=0.02; **Figure 3B**).
Based on the marked injection sites on the NOGA map, 50 myocardial segments were identified as located within the injected territory (injected segments), whereas 188 myocardial segments were located outside the injected territory (non-injected segments). As presented in **Table 2**, regional wall thickening in particular improved in injected myocardial segments, whereas no significant improvement was observed in non-injected myocardial segments.

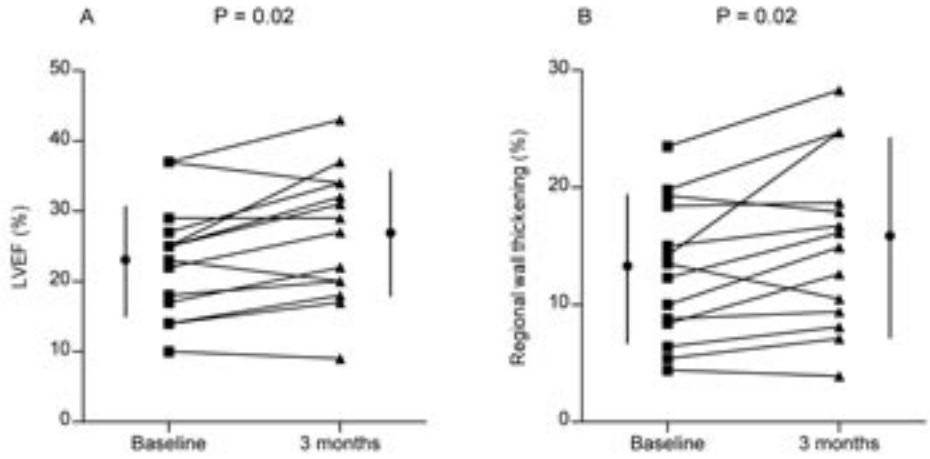

Figure 3
(A) Left ventricular ejection fraction (LVEF) and (B) regional wall thickening at baseline and 3 months after bone marrow cell injection as assessed by Tc-99m tetrofosmin gated SPECT.

Myocardial Perfusion

At 3 months, there was a trend towards an improved patients' segmental perfusion score (2.2±0.4 at baseline vs. 2.0±0.5 at 3 months; P=0.08). As presented in **Table 2**, injected myocardial segments displayed a significant improvement in myocardial perfusion. In contrast, the perfusion score remained unchanged in non-injected myocardial segments.

Myocardial Glucose Metabolism

Patients' segmental F18-FDG score tended to improve from 1.9±0.5 at baseline to 1.8±0.5 at 3 months (P=0.09). In injected segments, there was a trend toward an improved F18-

Table 2. Regional wall thickening, myocardial perfusion and myocardial glucose metabolism in injected and in non-injected segments.

Variable	Injected segments (n=50)	Non-injected segments (n=188)	P-value*
Regional wall thickening (%)			
Baseline	6.6±6.3	16.5±8.9	<0.01
3 months	11.7±7.0	19.1±9.1	0.01
Change from baseline, P-value†	<0.01	NS	
Myocardial perfusion score			
Baseline	3.5±0.7	1.8±0.4	<0.01
3 months	3.0±0.9	1.7±0.5	<0.01
Change from baseline, P-value†	0.02	NS	
Myocardial FDG score			
Baseline	2.9±0.9	1.7±0.4	<0.01
3 months	2.6±1.0	1.6±0.4	<0.01
Change from baseline, P-value†	0.06	NS	

*Assessed by unpaired Student's t-test; †Assessed by paired Student's t-test

FDG score (**Table 2**). In contrast, the F18-FDG score remained unchanged in non-injected segments. **Figure 4** displays a typical example of improved glucose metabolism in injected segments.

Discussion

The results of the present study demonstrate that intramyocardial bone marrow cell injection in patients with chronic myocardial infarction and severe LV dysfunction is safe and feasible in the short and midterm follow-up. In particular, the injection procedure was well tolerated and there were no periprocedural complications. Despite being a safety and feasibility study, the results of the present study also suggest that bone marrow cell injection in patients with chronic myocardial infarction and severe LV dysfunction is associated with a decrease in heart failure symptoms and an increased LV ejection fraction. In addition, a significant improvement in regional wall thickening and myocardial perfusion was observed in injected myocardial segments.

The results of the present study are in line with findings from 2 previous studies that investigated the efficacy of bone marrow cell transplantation in patients with chronic myocardial infarction.[10;11] In the present study, gated SPECT at 3 months demonstrated a 4% increase in LV ejection fraction. In the TOPCARE-CDH study, bone marrow cell transplantation in patients with chronic myocardial infarction was associated with a 3% increase in LV ejection fraction at 3 months.[10] Similarly, the IACT investigators reported an 8% increase in LV ejection fraction, whereas no improvement was observed in control patients.[11] In the IACT study, the increase in LV function was paralleled by a reduced perfusion defect size and increased myocardial glucose metabolism. Likewise, SPECT imaging in the present study showed an improved myocardial perfusion and a trend toward increased myocardial glucose metabolism in injected myocardial segments.

Although the main findings of the present study are in line with the results from the IACT and the TOPCARE-CHD study, this is the first study to evaluate bone marrow cell injection in patients with advanced postinfarction LV remodeling and a severely impaired LV function. The IACT and the TOPCARE-CDH studies included patients with chronic myocardial infarction, but patients with severe heart failure symptoms were excluded. In addition, LV ejection fraction in the IACT and TOPCARE-CDH study populations was remarkably preserved. In contrast, patients in the present study had severe heart failure symptoms (at least NYHA class III) and a severely depressed LV ejection fraction, with a mean of 23±8% at baseline.

Apart from a different study population, a different route for cell delivery was used in the present study. Whereas in the TOPCARE-CHD and IACT studies bone marrow cells were infused in the infarct-related artery, in the present study bone marrow cells were directly injected into the myocardium with the use of an endoventricular catheter guided by the NOGA system. This electromechanical mapping system was used to discriminate between

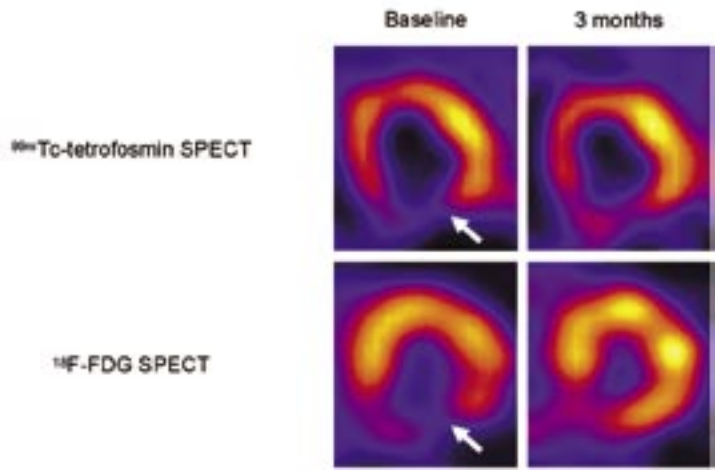

Figure 4
Tc-99m tetrofosmin and F18-FDG SPECT images at baseline and at 3 months follow-up showing an improvement in myocardial perfusion paralleled by an improvement in myocardial glucose metabolism in the inferior wall (arrow).

viable and non-viable myocardium and has the advantage that it allows specific targeting of the cell injections at the infarction border zone. Furthermore, direct intramyocardial injection may theoretically optimize cell engraftment in patients with chronic myocardial infarction because these patients are unlikely to release "homing" signals.[12;13] Finally, this injection technique allows cell delivery in myocardial regions with an occluded epicardial artery. Indeed, the majority of cell injections in the present study were targeted at myocardial sites with an occluded epicardial artery. The results of the present study demonstrate that NOGA-guided intramyocardial bone marrow cell injection in the infarction border zone is feasible and not associated with periprocedural complications in 15 subjects with chronic myocardial infarction and severe LV dysfunction. Future studies are warranted to identify the most optimal delivery route in patients with chronic myocardial infarction.

Study Limitations

The present study has several limitations. Because a control group was not included, the clinical improvement could be considered a placebo effect. In addition, the unblinded nature relative to certain end-points (e.g. hospital admissions) and exclusion of the patient who died during the study period from some analyses may have biased the results. Therefore, the present findings need confirmation in randomized, placebo-controlled studies comprising a large cohort of patients with chronic myocardial infarction and a severely depressed LV function.

Conclusions

The results of the current study illustrate that autologous bone marrow cell injection in patients with chronic myocardial infarction and severe LV dysfunction is safe and feasible in the short and midterm follow-up. In addition, bone marrow cell injection appears to be associated with a reduction in heart failure symptoms and an improved LV function. The excellent safety profile and the favorable effects on clinical parameters justify the performance of additional studies to further pursue intramyocardial bone marrow cell injection as a novel treatment strategy for patients with chronic myocardial infarction and severe LV dysfunction.

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