Cardiac bone marrow cell injection for chronic ischemic heart disease

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Chapter 6

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Usefulness of Intramyocardial Injection of Autologous Bone Marrow-derived Mononuclear Cells in Patients with Severe Angina Pectoris and Stress-induced Myocardial Ischemia

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Abstract

Introduction: Bone marrow cell transplantation has been proposed as a novel therapeutic option for patients with coronary artery disease. This study investigated whether autologous bone marrow-derived mononuclear cell injection into ischemic myocardium of patients with severe angina pectoris could safely reduce anginal symptoms, improve myocardial perfusion and increase left ventricular (LV) function.

Methods: In a total of 20 patients (63±10 years, 16 men) with angina pectoris, myocardial segments with stress-induced ischemia as assessed by single photon emission computed tomography (SPECT) were injected with 30 to 100 million mononuclear cells. Anginal symptoms, Canadian Cardiovascular Society (CCS) class, and quality of life were assessed at 3 and 6 months follow-up. At baseline and 3 months follow-up, an exercise test, SPECT and magnetic resonance imaging (MRI) were performed to assess exercise capacity, myocardial perfusion and LV function.

Results: Intramyocardial injection of autologous bone marrow-derived mononuclear cells was safe. The CCS class improved from 3.5±0.5 at baseline to 2.4±0.6 after 3 months ($P<0.01$) and 2.4±0.6 after 6 months ($P<0.01$). Quality of life improved from 52±10% to 71±10% at 3 months ($P<0.01$) and 73±15% at 6 months ($P<0.01$). Exercise capacity increased from 79±31% to 84±29% ($P<0.05$). MRI revealed an increased LV ejection fraction from 51±11% to 54±10% ($P<0.01$) and a reduced LV end-systolic volume from 97±50 ml to 88±42 ml ($P<0.01$). Wall motion score index improved from 0.36±0.32 to 0.24±0.28 ($P<0.01$). The number of segments with stress-induced ischemia reduced from 5.1±3.2 to 2.3±2.6 ($P<0.01$).

Conclusion: Autologous bone marrow-derived mononuclear cell injection in patients with ischemia is safe, reduces anginal symptoms, improves myocardial perfusion and increases LV function.

Introduction

Most clinical studies investigating autologous bone marrow cell transplantation as a treatment for ischemic myocardium have been performed in patients with acute myocardial infarction.[1-4] Data from patients with chronic myocardial ischemia and subsequent angina pectoris are rare. Only 2 previous studies with ≤ 10 patients assessed the safety and feasibility of autologous bone marrow cell injection in patients with angina pectoris and stress-induced ischemia.[5,6] The effectiveness of bone marrow cell injection in reducing stress-induced ischemia in patients with chronic coronary artery disease remains to be investigated. The aim of the present study was to evaluate the hypothesis that autologous bone marrow-derived mononuclear cell injection into the ischemic myocardium of patients with refractory angina pectoris could safely reduce anginal symptoms, improve myocardial perfusion and increase left ventricular (LV) function.

Methods

Patients

The study group consisted of patients with chronic coronary artery disease and ischemia on nuclear imaging (technetium-99m single photon emission computed tomography SPECT). Patients had severe angina despite maximally tolerated medical therapy at enrolment and were ineligible for percutaneous or surgical revascularization as assessed by coronary angiography. A committee comprising 2 cardiovascular surgeons and 2 interventional cardiologists determined the ineligibility for percutaneous or surgical revascularization. Twenty-three patients fulfilled the inclusion criteria and were considered as candidates for the study.

Exclusion criteria were a LV ejection fraction <40%, acute myocardial infarction within 6 months of enrolment in the study, a history of malignant disease, renal dysfunction (serum creatinine >200 µmol/L), or unexplained hematologic or biochemical abnormalities. The local ethics committee approved the protocol, and all patients gave informed consent.

Study Protocol

The baseline assessment included a clinical evaluation and a laboratory evaluation (complete blood count, blood chemistry, erythrocyte sedimentation rate, C-reactive protein, creatine kinase and troponin T serum levels). Patients kept record of their daily angina frequency and sublingual nitrate use. The severity of angina was graded according to the Canadian Cardiovascular Society (CCS) score. The Angina Seattle Questionnaire was used to assess quality of life. Within 2 weeks before the injection procedure an exercise bicycle test (to evaluate exercise capacity), 24-hour Holter monitoring (to assess ventricular arrhythmia), magnetic resonance imaging (MRI, to assess LV function, volumes and scar tissue) and SPECT (to assess perfusion and ischemia) were performed.

Follow-up evaluations performed 1, 3 and 6 months after the injection procedure consisted of a clinical evaluation, a laboratory evaluation and 24-hour Holter monitoring (to assess ventricular arrhythmia). At 3 and 6 months angina (CCS score) and quality of life (Seattle Angina Questionnaire) were assessed. At 3 months follow-up, an exercise bicycle test, MRI and SPECT were performed.

Bone Marrow Aspiration and Isolation of Mononuclear Cells

Bone marrow was harvested from the iliac crest under local anesthesia and placed in heparinized Hanks balanced salt solution. Mononuclear cells were isolated by Ficoll density gradient centrifugation according to Good Manufacturing Practice regulations, washed in phosphate buffered saline with 0.5% human serum albumin and resuspended in phosphate buffered saline with 0.5% human serum albumin. After concentration, the final suspension had a volume of 2 ml and a concentration of $15x10^6$ mononuclear cells/ml in the first 5 patients, and $50x10^6$ mononuclear cells/ml in the remaining 15 patients. The filtered bone marrow had no clots and stained negative for bacteria. The bone marrow cell population was analyzed by fluorescence-activated cell sorting using anti-CD34 and anti-CD45 antibodies (Becton Dickinson, Palo-Alto, California, USA). As a viability and quality ex vivo control, $10x10^6$ cells grown in culture medium (Fetal Bovine Serum and Dulbecco's Modified Eagle Medium-glucose with penicillin and streptomycin; Invitrogen, Eugene, Oregon, USA) were found to be able to generate mesenchymal cells in culture.

Intramyocardial Injection of Mononuclear Bone Marrow-Derived Cells

During isolation of the bone marrow cells, patients underwent non-fluoroscopic LV electromechanical mapping with the NOGA system (NOGAstar catheter, Biosense-Webster, Waterloo, Belgium) to guide the injections of bone marrow cells to the area of ischemic myocardium as assessed on SPECT. All patients received an intravenous dose of 7500 U heparin. After completion of LV mapping, the mapping catheter was replaced by an injection catheter (MyoStar catheter, Biosense-Webster), from which a 27-gauge injection needle can be advanced by 4-6 mm for direct intramyocardial injection. The general region for treatment was selected by matching the ischemic area identified on SPECT. The electromechanical map was then used to target the specific treatment area by identifying viable myocardium (unipolar voltage ≥6.9 mV)[7] within that region. The injection catheter was prepared as described previously.[8] Before every injection of cells into the LV myocardium, the following criteria had to be met: perpendicular position of the catheter to the myocardial wall, excellent loop stability (<4 mm) underlying voltage ≥6.9 mV, and the presence of a premature ventricular contraction on extension of the needle into the myocardium. Subsequently, 8-12 intramyocardial injections of approximately 0.2 ml each were delivered.

Periprocedural Evaluation

Patients had serum C-reactive protein, erythrocyte sedimentation rate, complete blood count, and creatine kinase levels measured and an electrocardiogram performed just before the procedure. Immediately after the procedure, another electrocardiogram was performed and 2 day continuous heart rhythm monitoring was started. Complete blood count, serum C-reactive protein, erythrocyte sedimentation rate, creatine kinase and troponin T levels were assessed immediately after the procedure, and after 6, 24 and 48 hours. Patients were monitored in the cardiac intensive care unit for 24 hours after the injection procedure. Before discharge, 3-4 days after the injection procedure, 2D echocardiography was performed to exclude pericardial effusion.

Assessment of Exercise Capacity

All patients performed a symptom-limited bicycle exercise test with a 20 Watt starting load and 10 Watt increments per minute before and 3 months after the injection procedure. All anti-anginal medication was continued. Test end points were angina pectoris, physical exhaustion, dyspnea, significant decrease in blood pressure (>10 mmHg), or achievement of maximal age-related heart rate. A 12-lead electrocardiogram was recorded before, during and after the test. Total exercise duration, maximal workload achieved in percentage (expected for age, gender, length and weight) and the time to significant ST-segment changes were compared before and 3 months after bone marrow-derived mononuclear cell injection.

Magnetic Resonance Imaging

MRI studies at baseline and 3 months follow-up were performed as previously described.[9] A 1.5 Tesla MRI system (Philips Medical Systems, Best, The Netherlands) with 5-segment synergy coil and vector electrocardiographic gating was used. The entire heart was imaged in the short axis view during multiple 15 second breath holds, using a steady state free precession (field of view 400x400 mm^2, matrix size 256x256). Contrast enhanced images (to assess scar tissue) were acquired 15 minutes after gadolinium-diethylenetriamine pentaacetic acid administration (0.15 mmol/kg, Magnevist, Schering AG/Berlex Laboratories, Berlin, Germany).

LV function and volumes were calculated using the MR Analytical Software System (MASS, Medis, Leiden, The Netherlands). To determine the regional wall motion at rest, cine MRI images were visually interpreted by 2 experienced observers (unaware of other MRI and clinical data) using a previously described 17-segment model.[10] Each segment was assigned a wall motion score using a 5-point scale (0=normokinesia, 1=mild hypokinesia, 2=severe hypokinesia, 3=akinesia, 4=dyskinesia).[11] The patients' segmental scores were summed and divided by 17 to yield the summed wall motion score index (reflecting LV function).

Contrast-enhanced images were scored by 2 experienced observers (unaware of other MRI

and clinical data) using the same 17-segment scoring system. Each segment was graded on a 5-point scale (0=absence of hyperenhancement, 1=hyperenhancement 1% to 25% of LV wall thickness, 2=hyperenhancement extending to 26% to 50% of LV wall thickness, 3=hyperenhancement extending to 51% to 75%, 4= hyperenhancement extending to 76% to 100% of the LV wall thickness).12 The number of affected segments was considered to reflect the spatial (circumferential) extent of scar tissue. The number of segments with a segmental scar score of 3 or 4 was considered to reflect the transmurality of scar tissue in the infarct zone. Patients' segmental scores were summed and divided by 17 to yield the total scar score (reflecting the total scar per patient).

SPECT Imaging

For the SPECT examination a two-day stress-rest protocol was used. The stress protocol included a symptom-limited bicycle exercise test. Technetium-99m tetrofosmin (500 MBq) was injected intravenously at peak exercise, which was continued for 1 minute after tracer injection. In patients unable to perform physical exercise, adenosine (n=8) or dobutamine (n=2) was used. On the second day, resting images were obtained (using 500 MBq technetium-99m tetrofosmin) after the patient's daily dose of nitrates. Imaging was performed with a triple-head SPECT camera (GCA 9300/HG, Toshiba Corp., Tokyo, Japan) as previously described.[13]

Reconstruction yielded long- and short-axis projections perpendicular to the heart axis. The short-axis slices were displayed in polar map format, adjusted for peak myocardial activity (100%). The myocardium was divided into 17 segments, similar to the MRI studies.[10]

Segmental tracer activity was expressed as a percentage of the maximum on a 4-point scale: 1=normal tracer activity >75%; 2=tracer activity 50-75%; 3=tracer activity 25-50%; 4=tracer activity <25%. Perfusion defects on stress images were considered present when tracer activity was <75% of maximum. When significant fill-in (>10%) of perfusion defects was observed on the resting images, segments were classified as ischemic. Summation of the patients' segmental scores at rest yielded the summed rest score and summation of the patients segmental stress scores yielded the summed stress score.

Statistical Analysis

Data are reported as mean ± SD. Quantitative data were compared using paired, two-tailed Student's t test. Categorical data were compared using Wilcoxon signed ranks test. A P-value <0.05 was considered significant.

Results

A total of 20 patients were included. The baseline clinical characteristics are listed in **Table 1**. All patients had triple vessel disease and were ineligible for conventional revascularization, because they had diffuse coronary artery disease with no epicardial artery suitable for surgery or angioplasty. During the 6 months follow-up period, the type and dose of medications remained unchanged.

Procedural Data

The total procedural time for mapping and injection was 59±19 minutes. The electromechanical maps comprised an average of 90±22 points. Patients received 10±2 injections of 0.2 ml each in segments with stress-inducible ischemia. The injected cell suspension contained 41±16x10^6/ml bone marrow-derived mononuclear cells. The CD34/CD45-positive cell fraction was 2.5±1.6%.

Table 1. Clinical characteristics of the study population (n=20)

Characteristic		
Age (years)	63±10	
Men	16	(80 %)
Systemic hypertension	9	(45 %)
Diabetes mellitus	11	(55 %)
Insulin dependent	6	(30 %)
Non-insulin dependent	5	(25 %)
Hyperlipidemia (total cholesterol > 5 mmol/L)	17	(85 %)
Smoker	2	(10 %)
Coronary artery disease in family	13	(65 %)
Body mass index (Kg\m^2)	28±5	
Prior myocardial infarction	14	(70 %)
Prior percutaneous coronary intervention	17	(85 %)
Prior coronary artery bypass grafting	19	(95 %)
Current medication		
Nitrates	20	(100 %)
β-blockers	18	(90 %)
Calcium channel blockers	15	(75 %)
Statins	19	(95 %)
Angiotensin-converting enzyme inhibitors	14	(70 %)
Number of anti-anginal medications (1/2/3)	0 / 7 / 13	(0 / 35 / 65 %)
Number of daily anginal episodes	2.8±4.1	
Exercise duration (minutes)	7.5±3.9	
Time to ischemia (minutes)	5.0±2.9	

Safety Data

Autologous bone marrow-derived mononuclear cell injection was safe. Periprocedural laboratory evaluations demonstrated an absence of inflammation or myocardial infarction (maximum erythrocyte sedimentation rate 21±15 mm; maximum C-reactive protein 23±21 mg/L; maximum creatine kinase 140±66 U/L; maximum Trop T 0.28±0.21 µg/L). Ventricular arrhythmia were not observed during injection or hospitalization. Postprocedural pericardial effusion was excluded on 2D echocardiography before discharge. Patients were discharged 4±2 days after the injection procedure. Twenty-four hour Holter recordings 1, 3 and 6 months after injection did not reveal ventricular arrhythmia. No patients were readmitted to the hospital during the 6 months follow-up period.

Clinical Outcome

The frequency of angina episodes per day decreased from 2.8±4.1 at baseline to 1.0±1.5 at 3 months (P<0.01) and 0.6±1.2 at 6 months (P<0.05). Similarly, the frequency of sublingual use of nitrates per day decreased from 1.8±2.8 tablets/day at baseline to 0.5±1.2 tablets/day at 3 months (P<0.01) and 0.5±1.3 tablets/day at 6 months follow-up (P<0.01). In 2 patients, no clear improvement in anginal symptoms occurred. The mean CCS score improved from baseline to 3 months and remained unchanged at 6 months (**Figure 1**). Quality of life improved from 52±10% to 71±10% at 3 months (P<0.01) and 73±15% at 6 months (P<0.01). In particular, significant improvements were found in all 5 scales of the Seattle Angina Questionnaire at 3 and 6 months when compared to baseline (**Figure 2**).

Exercise Capacity

The maximal workload achieved increased from 79±31% to 84±29% at 3 months follow-up (P<0.05). A trend towards an increased mean exercise duration from 7.5±3.9 to 8.0±3.4 min at 3 months was found, although the difference was not significant (P=0.07). The

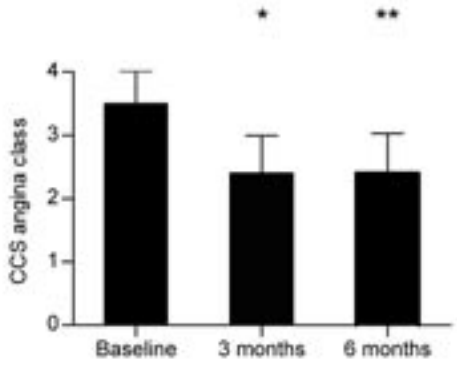

Figure 1

Mean Canadian Cardiovascular Society (CCS) angina class at baseline, 3 months and 6 months after bone marrow-derived mononuclear cell injection. CCS angina class improved from 3.5±0.5 to 2.4±0.6 at 3 months (*: P<0.01) and 2.4±0.6 at 6 months follow-up (**: vs. baseline P<0.01 and P>0.05 vs. 3 months).

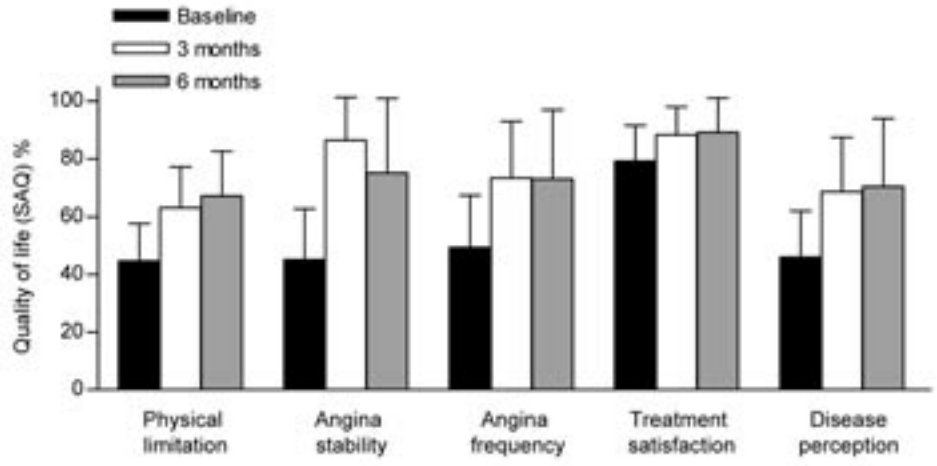

Figure 2
Quality of life assessment using the Seattle Angina Questionnaire (SAQ) showed significant improvements in all five scales at 3 (white bars, all P<0.01) and 6 months (grey bars, all P<0.01) compared with baseline (black bars). Data presented as mean ± SD.

number of patients who experienced angina during the test reduced from 16/20 (80%) to 8/20 (40%) at 3 months follow-up (P<0.01). At baseline, in 11/20 (55%) patients significant ST-segment depression was recorded compared with 10/20 (50%) patients at 3 months follow-up (P=NS). The time to significant ST-segment depression increased from 5.0±2.9 minutes at baseline to 6.1±3.2 minutes at 3 months follow-up (P<0.05).

Left Ventricular Function and Scar Tissue Assessed by MRI

Because of severe obesity, MRI was not feasible in 1 patient. The MRI findings are presented in **Table 2** and **Figure 3**. At 3 months follow-up, the LV ejection fraction increased significantly compared with baseline. The LV end-systolic volume decreased compared with baseline, whereas the LV end-diastolic volume remained unchanged.
Regional LV function had improved significantly at 3 months follow-up as demonstrated

Table 2. Magnetic resonance imaging findings

Variable	Baseline	3 months	P-Value
Left ventricular ejection fraction (%)	51±11	54±10	<0.01
Left ventricular end-systolic volume (ml)	97±50	88±42	<0.01
Left ventricular end-diastolic volume (ml)	189±63	186±58	NS
Wall motion score index	0.36±0.32	0.24±0.28	<0.01
No. of dysfunctional segments	3.84±2.65	2.32±2.19	<0.01
No. of normally contracting segments	13.2±2.7	14.7±2.2	<0.01
Spatial extent of scar tissue	4.64±2.87	4.21±3.09	NS
Transmurality score	1.07±1.59	1.07±1.33	NS
Total scar score	0.48±0.39	0.45±0.39	NS

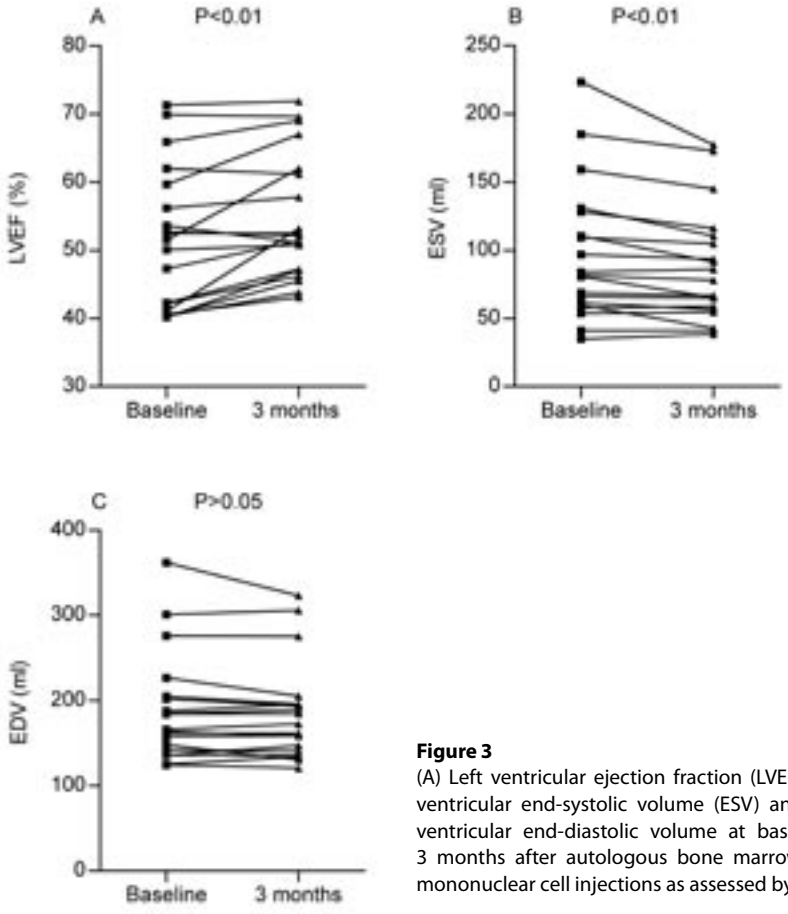

Figure 3
(A) Left ventricular ejection fraction (LVEF), (B) left ventricular end-systolic volume (ESV) and (C) left ventricular end-diastolic volume at baseline and 3 months after autologous bone marrow-derived mononuclear cell injections as assessed by MRI.

by the decreased wall motion score index. The number of dysfunctional segments per patient decreased significantly, and the number of normokinetic segments increased after bone marrow-derived mononulear cell injection.
Contrast-enhanced images were obtained in 18 patients at baseline and 3 months follow-up. Fourteen patients had areas of hyperenhancement (indicating scar tissue) on MRI at baseline. Total scar score, transmurality score and spatial extent of scar tissue remained unchanged at 3 months follow-up.

Myocardial Perfusion by SPECT

At 3 months follow-up, the number of segments with stress-inducible ischemia per patient decreased from 5.1±3.2 to 2.3±2.6 (P<0.01). A typical example of SPECT polar maps before and 3 months after autologous bone marrow-derived mononuclear cell injections is shown in **Figure 4**. The summed stress score per patient improved from 25.6±5.0 to 21.6±4.2

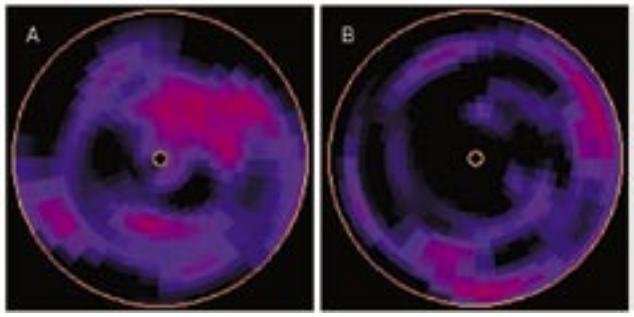

Figure 4
Example of SPECT polar maps at (A) baseline and (B) 3 months follow-up showing profound reduction in extent of stress-inducible ischemia.

(P<0.01). Also, perfusion at rest improved, as evidenced by an increase in summed rest score from 19.8±4.1 to 19.2±3.9 (P<0.05).

Discussion

The results of this study have illustrated that autologous bone marrow-derived mononuclear cell injection in patients with angina pectoris and stress-inducible ischemia could reduce anginal symptoms and improve exercise capacity without occurrence of ventricular arrhythmia. This is the first study to demonstrate, in patients with chronic ischemia without severely impaired LV function, that bone marrow cell injection enhances myocardial perfusion and improves systolic LV function.

Improvement of LV ejection fraction at 3 months follow-up was most likely a result of increased myocardial perfusion and improved regional wall motion. This resulted in a reduced LV end-systolic volume; the LV end-diastolic volume did not decrease. Consequently, the therapeutic effect appears more related to enhanced myocardial contractility rather than induction of LV reverse remodelling. Three months after bone marrow cell injection, the amount of myocardium exhibiting delayed enhancement on contrast-enhanced MRI remained unchanged. This observation suggests that conversion of scar tissue into viable myocardium or embolization of ischemic myocardium could not have contributed to the reported effects. The findings of the present study are consistent with the hypothesis that bone marrow cells promote angiogenesis, resulting in increased myocardial perfusion.[14] The promotion of angiogenesis could be caused by differentiation of bone marrow cells in endothelial and/or vascular smooth muscle cells[15] or by the production of angiogenic cytokines, as previously proposed.[14]

Bone marrow cell transplantation has been proposed as a novel therapeutic option for patients with coronary artery disease. Until now, most clinical studies investigating autologous bone marrow cell transplantation were performed in patients with acute myocardial infarction.[1-4] Data from patients with chronic ischemia are scarce. At present,

only 3 studies of patients with chronic coronary artery disease have been published.[5,6,16] The current results are in line with those of 2 studies on the safety of intramyocardial bone marrow cell transplantation in patients with chronic ischemia and angina pectoris. Tse et al. described a reduction in anginal symptoms, improved wall motion and improved wall thickening at 3 months follow-up in 8 patients.[6] In addition, the area of hypoperfused myocardium was reduced on MRI. Fuchs et al. reported a reduction in anginal symptoms and improved myocardial perfusion in 10 patients with a trend toward improved LV ejection fraction.[5] The present study has confirmed the previously suggested beneficial effects on angina, myocardial perfusion and regional wall motion, but it is the first to demonstrate an increased LV ejection fraction in this patient group.

The currently described increase in LV ejection fraction is in line with the improvement in LV ejection fraction reported in patients with heart failure[8] and patients with acute myocardial infarction.[1-3] However, it extends these observations to patients with chronic ischemia without severely impaired LV function. The finding that the improved LV ejection fraction is mainly based on improved myocardial perfusion[3,4,8] and a reduced LV end-systolic volume is consistent with earlier studies.[2,4,8]

The major limitation of the current study was the lack of a randomized control group. Consequently, the described beneficial effects could have been attributable to the placebo effect. However, all clinical, SPECT, and MRI data were analyzed by two investigators who were unaware of all other clinical and imaging data.

Obviously, the present study was not designed to assess the underlying cellular mechanism of bone marrow cell injection improving myocardial perfusion and LV function. Therefore, secretion of pro-angiogenic factors by the bone marrow cells and differentiation of bone marrow cells in endothelium cells, smooth muscle cells or cardiomyocytes could have contributed to the described effect. In future studies, cell tracking by labeling of injected cells will provide more mechanistic insights.

Conclusions

In summary, the results of the current study document that intramyocardial autologous bone marrow-derived mononuclear cell injection in patients with angina pectoris and ischemia was safe, improved anginal symptoms, enhanced myocardial perfusion and increased LV function. These encouraging results provide rationale for randomized, double blind, placebo-controlled trials in patients with chronic coronary artery disease.

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