Cardiac bone marrow cell injection for chronic ischemic heart disease

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Chapter 5

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Intramyocardial Bone Marrow Cell Transplantation and the Progression of Coronary Atherosclerosis in Patients with Chronic Myocardial Ischemia

Submitted

Abstract

Introduction: Cell therapy has been proposed as a novel treatment strategy for patients with ischemic heart disease. However, 2 recent studies suggested that cardiac cell transplantation may aggravate coronary atherosclerosis. The aim of the current study was to assess whether intramyocardial bone marrow cell transplantation in patients with chronic myocardial ischemia is associated with progression of coronary atherosclerosis.

Methods: In 30 patients with chronic ischemia bone marrow was aspirated from the iliac crest. During mononuclear cell isolation, coronary angiography was performed. Thereafter, $94\pm18x10^6$ cells were injected intramyocardially (NOGA system) in regions with ischemia on Tc-99m tetrofosmin SPECT.

Results: During the 12 months follow-up period there was no clinical evidence of progression of coronary atherosclerosis. CCS class improved from 3.4±0.5 to 2.4±0.8 at 3 months, 2.4±0.9 at 6 months and 2.5±0.9 at 12 months ($P<0.01$). Magnetic resonance imaging-determined left ventricular ejection fraction increased from 51±12% to 54±12% at 3 months ($P<0.01$) and the number of ischemic segments per patient on Tc-99m tetrofosmin SPECT decreased from 5.2±2.6 to 2.1±2.2 at 3 months ($P<0.01$). Repeat coronary angiography at 4 months revealed that bone marrow cell transplantation did not decrease minimal luminal diameter (1.81±0.80 mm vs. 1.79±0.82 mm; P=NS) or mean luminal diameter (2.48±0.85 mm vs. 2.46±0.86 mm; P=NS). Similarly, the percentage diameter stenosis (32±19% vs. 32±20%; P=NS) and the atheromatosis severity score (4.78±2.40 vs. 4.80±2.40; P=NS) remained unchanged.

Conclusion: Intramyocardial bone marrow cell transplantation in patients with chronic myocardial ischemia was not associated with significant progression of coronary atherosclerosis.

Introduction

Cell transplantation has been proposed as a novel treatment strategy for patients with ischemic heart disease. Animal model studies demonstrated that cardiac bone marrow cell transplantation is associated with increased vascularization and improved left ventricular systolic function.[1-4] On the basis of the encouraging pre-clinical findings, there is a growing number of human studies investigating the safety, feasibility and efficacy of this novel treatment strategy in patients with acute myocardial infarction or chronic myocardial ischemia. From these initial clinical studies it appears that cardiac bone marrow cell transplantation is feasible and may beneficially affect myocardial perfusion and left ventricular function.[5-14]

However, concern has recently been raised regarding the safety of cell therapy as 2 studies in patients with acute myocardial infarction suggested that bone marrow cell transplantation may aggravate coronary atherosclerosis. The MAGIC study indicated that intracoronary infusion of granulocyte colony-stimulating factor (G-CSF) mobilized cells was associated with increased restenosis rates in post-myocardial infarction patients.[13] Similarly, Bartunek et al. reported a higher incidence of coronary events in patients receiving intracoronary infusion of CD133+ enriched bone marrow cells as compared to control patients.[8]

At present the effect of cardiac bone marrow cell transplantation on the progression of atherosclerosis in patients with chronic myocardial ischemia has not been evaluated. Therefore, the aim of the current study was to explore whether bone marrow cell transplantation in patients with chronic myocardial ischemia is associated with progression of coronary artery atherosclerosis as assessed by quantitative coronary angiography.

Methods

Patients

The study group consisted of patients with chronic coronary artery disease scheduled for intramyocardial bone marrow cell injection. Patients had refractory angina (Canadian Cardiovascular Society (CCS) class III or IV) despite optimal medical therapy and stress-inducible ischemia on Tc-99m tetrofosmin single photon emission computed tomography (SPECT). All patients were not eligible for conventional revascularization due to poor target vessels on recent (<6 months) coronary angiography. A committee of 2 cardiovascular surgeons and 2 interventional cardiologists determined the ineligibility for percutaneous or surgical revascularization.

Patients with a recent myocardial infarction (<6 months), a history of malignancy, renal dysfunction (serum creatinine >200 µmol/L), a cardiac pacemaker or unexplained hematological or biochemical laboratory abnormalities were excluded. The study protocol was approved by the local ethics committee and written informed consent was obtained from all patients.

Study Protocol

Within 2 weeks before cell injection, stress-rest Tc-99m tetrofosmin SPECT was performed to identify the location of stress-inducible myocardial ischemia and magnetic resonance imaging was performed to assess left ventricular function. At hospital admission for cell injection, an independent physician scored the CCS functional class.

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) a coronary angiogram was obtained. Thereafter, a 3D electromechanical map of the left ventricle was made with the use of the NOGA system (Biosense-Webster, Waterloo, Belgium).[15,16] Intramyocardial cell injections were targeted at myocardial regions with stress-inducible ischemia (on SPECT) and injected with the NOGA system as previously described.[17] Immediately after the injection procedure continuous heart rhythm monitoring was started for 2 days. Before discharge, 2D echocardiography was performed to exclude post-procedural pericardial effusion.

The severity of angina (CCS class) was reassessed at the outpatient clinic by an independent physician at 3, 6 and 12 months follow-up. Magnetic resonance imaging and stress-rest Tc-99m tetrofosmin SPECT were repeated at 3 months to re-evaluate left ventricular function and myocardial perfusion. A follow-up coronary angiogram was obtained at 4 months follow-up to assess the progression of atherosclerosis. Cardiovascular medication remained unchanged during the 12 months follow-up period.

Coronary Angiography

Coronary angiography was performed according to standard clinical protocols. To obtain vascular access, the femoral approach using the Seldinger technique was applied. Angiographic recordings were preceded by intracoronary injections of 300 µg of nitroglycerine. For each coronary segment, at least 2 orthogonal projections were filmed. The precise rotational and angulational views of the initial coronary angiogram were noted. The projections were duplicated precisely at follow-up angiography. Digital images were stored on compact disk in DICOM format.

For quantitative coronary angiography, segmentation of the coronary arteries was performed on the basis of the American Heart Association/American College of Cardiology guidelines.[18] Segments that were opacified solely by collateral circulation (generally distal to total occlusions) were excluded from the analysis (n=54). Each coronary segment was analyzed quantitatively in 2 orthogonal views if both projections were free of significant foreshortening. Quantitative coronary angiography was carried out by a blinded investigator using the Cardiovascular Measurement System (CMS-QCA 6.0, MEDIS Medical Imaging Systems, Leiden, The Netherlands) as previously described.[19] In brief, the contrast-filled non-tapered catheter tip was used for calibration. To determine the contours of the vessel, the beginning and end of the coronary segment were indicated, after which a path line was computed connecting these 2 points. The boundaries of the selected segment

were detected automatically and adjusted manually when necessary. Subsequently, the following parameters were derived: segment length, reference diameter, minimal luminal diameter, mean luminal diameter and the percentage diameter stenosis. In addition, each coronary segment was assigned a segmental atheromatosis severity score (0 to 9) summarizing the mean severity of lesions within that segment.[20] For each parameter, the average values obtained in both views were calculated.

Magnetic Resonance Imaging

A 1.5-T Gyroscan ACS-NT magnetic resonance imaging scanner (Philips Medical Systems, Best, The Netherlands) equipped with powertrack 6000 gradients and 5-element cardiac synergy coil was used. Patients were positioned in the supine position. Images were acquired during breath holds of approximately 15 seconds using vector electrocardiographic gating. The heart was imaged from apex to base,[21] with 10 to 12 imaging levels (dependent on the heart size) in the short-axis view using a balanced, fast-field echo sequence with parallel imaging (SENSE, acceleration factor 2). Typical parameters were a field of view of 400 x 400 mm^2, matrix of 256 x 256 pixels, slice thickness of 10 mm, no slice gap, flip angle of 50^0, time to echo of 1.82 ms, and time to repeat of 3.65 ms. Temporal resolution was 25 to 39 ms.

An independent observer blinded to all clinical data (including the time point of the study) analyzed the magnetic resonance images. To determine parameters of global systolic function, endocardial borders were outlined manually on short-axis cine images with previously validated software (MASS, Medis, the Netherlands).[22] Papillary muscles were regarded as part of the left ventricular cavity, and epicardial fat was excluded. Left ventricular end-systolic and end-diastolic volumes were calculated. Stroke volume was the difference between end-diastolic and end-systolic volume. Left ventricular ejection fraction was stroke volume divided by end-diastolic volume.

SPECT Imaging

A previously described two-day stress-rest protocol was used for Tc-99m tetrofosmin SPECT imaging.[12] For stress imaging, pharmacological stress (intravenous administration of adenosine 0.14 mg/kg/min for 6 minutes (n=28), or dobutamine up to a maximum dose of 40 µg/kg/min in 15 minutes (n=2)) was used. One minute before termination of the test, 500 MBq Tc-99m tetrofosmin was injected intravenously. On the second day, resting images were obtained using 500 MBq Tc-99m tetrofosmin. Imaging was performed with a triple-head SPECT camera system (GCA 9300/HG, Toshiba Corp., Tokyo, Japan). Stress imaging was performed with the same type of stressor at baseline and at 3 months follow-up. An experienced observer who was blinded to all clinical data reviewed the SPECT images.

Stress and rest perfusion was analyzed quantitatively (17-segment model)[23] with the use of quantitative gated SPECT (QGS)-software (Cedars-Sinai Medical Center, Los Angeles,

California, USA).[24] When tracer uptake at stress was ≥75% of maximum tracer activity, segments were considered to be normally perfused. Segments with <75% tracer uptake at stress in which tracer uptake increased >10% at rest were considered to be ischemic.

Statistical Analysis

Continuous data are presented as mean±SD; categorical data are presented as numbers and percentages. Comparison of continuous data was performed using the paired and unpaired Student's t-test when appropriate. Categorical data were compared using Chi-square analysis. All statistical tests were two-sided, with a P-value <0.05 considered significant.

Results

Patient Characteristics

Thirty consecutive patients (26 men, age 64±10 years) were included in the study. The baseline characteristics are summarized in **Table 1**. As per protocol, all patients had severe angina pectoris with 18 patients in CCS class III and 12 patients in CCS class IV. Cardiovascular medication included long-acting nitrates (100%), β-blockers (93%), calcium channel blockers (83%), statins (100%), angiotensin-converting enzyme inhibitors (70%), acetylsalic acid (77%) and oral anti-coagulants (23%). During the 12 months follow-up period the type and dosages of medications remained unchanged. In addition, blood samples taken at baseline and at 4 months follow-up revealed that the serum total cholesterol (4.4±1.1 mmol/L vs. 4.4±1.2; P=NS), serum LDL (2.5±1.1 mmol/L vs. 2.4±1.2 mmol/L; P=NS) and serum HbA1c in diabetics (7.7±1.9% vs 7.8±2.0%; P=NS) remained unchanged.

Bone Marrow Cell Injection

The total time for mapping and cell injection was 55±18 minutes. The electromechanical map comprised an average of 92±20 points. In total, the final preparation of injected cells contained 94±18x10^6 mononuclear cells. Cell viability was 97±2% and the CD34+ cell fraction was 2.4±1.4%. Patients received 10±2 injections of approximately 0.2 ml each in regions with stress-inducible ischemia. No periprocedural complications occurred. In particular, repetitive laboratory measurements revealed no myocardial infarction, sustained ventricular tachycardia were not observed and 2D echocardiography did not reveal pericardial effusion.

Clinical Follow-up

During the 12 months follow-up period no major adverse event occurred: there were no deaths and there was no clinical evidence of progression of atherosclerosis. In particular, none of the patients experienced a myocardial infarction or underwent a coronary revascularization procedure. Moreover, assessment of the patients' clinical status revealed

Table 1. Clinical characteristics of the study population (n=30)

Characteristic		
Age (years)	64±10	
Gender (Men)	26	(87 %)
Cardiovascular risk factors		
Coronary artery disease in family	22	(73 %)
Systemic hypertension	13	(43 %)
Hypercholesterolemia	26	(87 %)
Current smoker	2	(7 %)
Diabetes mellitus	15	(50 %)
Insulin dependent	9	(30 %)
Non-insulin dependent	6	(20 %)
Cardiovascular history		
Prior myocardial infarction	22	(73 %)
Prior percutaneous coronary intervention	20	(67 %)
Prior coronary artery bypass grafting	28	(93 %)
Extent of coronary artery disease		
1-vessel disease	0	(0 %)
2-vessel disease	10	(33 %)
3-vessel disease	20	(67 %)

a significant reduction in anginal symptoms as mean CCS class improved from 3.4±0.5 at baseline to 2.4±0.8 at 3 months, 2.4±0.9 at 6 months and 2.5±0.9 at 12 months (P<0.01). The individual data are presented in **Figure 1**.

Magnetic resonance imaging at 3 months follow-up demonstrated a modest improvement in left ventricular function. As illustrated in **Figure 2**, left ventricular ejection fraction increased from 51±12% at baseline to 54±12% at 3 months follow-up (P<0.01). Stroke volume increased from 98±20 ml to 106±28 ml at 3 months (P<0.01). The increase in stroke volume was mainly related a decrease in left ventricular end-systolic volume (107±64 ml vs. 98±57 ml at 3 months; P=0.03), whereas left ventricular end-diastolic volume remained unchanged (205±75 ml vs. 204±72 ml; P=NS).

Tc-99m tetrofosmin SPECT imaging revealed that the number of ischemic segments per patient decreased from 5.2±2.6 at baseline to 2.1±2.2 at 3 months follow-up (P<0.01).

Angiographic Follow-up

In total, 367 coronary segments (with a mean of 12±2 per patient) were measured quantitatively and included in the analysis. At baseline, 309 (84%) segments had a diameter stenosis ≤50%, whereas in 58 (16%) segments the diameter stenosis was >50%. Coronary angiography results are summarized in **Table 2**. Reference diameter was 2.64±0.88 mm at baseline and 2.63±0.89 mm at 4 months follow-up (P=NS). Compared with baseline, bone marrow cell injection did not significantly decrease minimal luminal diameter (1.81±0.80

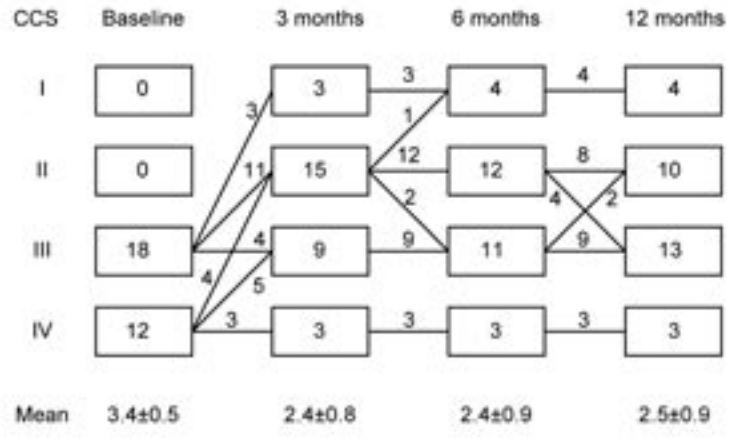

Figure 1
Individual CCS scores at baseline, at 3, at 6 and at 12 months follow-up. Mean CCS class improved from 3.4±0.5 at baseline to 2.4±0.8 at 3 months, 2.4±0.9 at 6 months and 2.5±0.9 at 12 months (P<0.01).
CCS = Canadian Cardiovascular Society

mm vs. 1.79±0.82 mm at 4 months; P=NS) or mean luminal diameter (2.48±0.85 mm vs. 2.46±0.86 mm at 4 months; P=NS). Likewise, the percentage diameter stenosis (32±19% vs. 32±20% at 4 months; P=NS) and the atheromatosis severity score (4.78±2.40 vs. 4.80±2.40 at 4 months; P=NS) remained unchanged.

When the analysis was restricted to coronary segments with a baseline diameter stenosis >50% (n=58), similar results were obtained. In particular, the reference diameter (2.07±0.78 vs. 2.06±0.77; P=NS), minimal luminal diameter (1.27±0.61 vs. 1.26±0.63, P=NS), mean luminal diameter (1.66±0.72 vs. 1.68±0.75; P=NS), percentage diameter stenosis (61±22% vs. 62±20%; P=NS) and atheromatosis severity score (6.45±1.99 vs. 6.48±2.00; P=NS) of the "diseased" coronary segments remained unchanged.

Angiographic Follow-up: Coronary Segments Within vs. Outside Injected Territory

By comparing the marked injection sites on the NOGA map and the individual coronary anatomy, 97 coronary segments were identified as located within the injected territory whereas 270 coronary segments were located outside the injected territory. If there was an overlap between coronary segments and areas of blood supply, all coronary segments that supplied blood to the injected region were classified as located within the injected territory.

Segments located within the injected territory had a significantly smaller reference diameter, minimal luminal diameter and mean luminal diameter as compared to segments located outside the injected territory (**Table 3**). Similarly, the atheromatosis severity score was significantly higher in segments located within the injected territory.

At 4 months, no significant change in reference diameter, minimal luminal diameter, mean luminal diameter, percentage diameter stenosis and atheromatosis severity score was noted in either group. The absolute change in reference diameter (+0.01±0.30 mm vs.

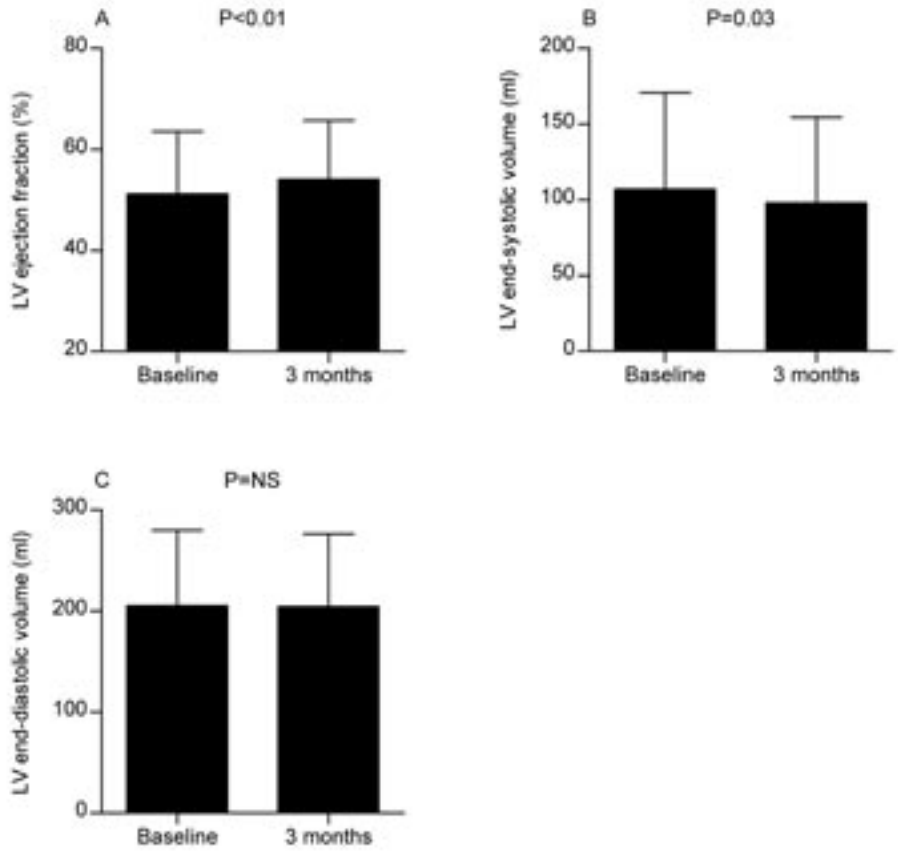

Figure 2
LV ejection fraction (A), LV end-systolic volume (B) and LV end-diastolic volume (C) at baseline and 3 months after bone marrow cell injection as assessed by magnetic resonance imaging.

-0.02±0.31 mm; P=NS), minimal luminal diameter (-0.03±0.26 mm vs. -0.01±0.28 mm; P=NS), mean luminal diameter (-0.02±0.20 mm vs. -0.03±0.28 mm; P=NS), percentage diameter stenosis (+2±14% vs. +1±11%; P=NS) and atheromatosis severity score (+0.02±1.83 vs. +0.01±1.44; P=NS) during the 4 months follow-up period was similar in both groups.

Table 2. Coronary angiography results (367 segments)

Variable	Baseline	4 months	P-value*
Segment length (mm)	29.4±13.9	29.3±13.9	NS
Reference diameter (mm)	2.64±0.88	2.63±0.89	NS
Minimal luminal diameter (mm)	1.81±0.80	1.79±0.82	NS
Mean luminal diameter (mm)	2.48±0.85	2.46±0.86	NS
Diameter stenosis (%)	32±19	32±20	NS
Atheromatosis severity score	4.78±2.40	4.80±2.40	NS

* Assed by paired student's t-test

Table 3. Coronary angiography results in coronary segments located within vs. coronary segments located outside the injected territory

Variable	Segments within injected territory (n=97)	Segments outside injected territory (n=270)	P-value*
Segment length (mm)			
Baseline	31.3±13.3	28.7±14.0	NS
4 months	31.3±13.1	28.6±14.1	NS
Change from baseline	+0.1±4.5	-0.1±4.7	NS
Change from baseline, P-value†	NS	NS	
Reference diameter (mm)			
Baseline	2.38±0.63	2.74±0.94	<0.01
4 months	2.38±0.67	2.72±0.94	<0.01
Change from baseline	+0.01±0.30	-0.02±0.31	NS
Change from baseline, P-value†	NS	NS	
Minimal luminal diameter (mm)			
Baseline	1.59±0.51	1.89±0.87	<0.01
4 months	1.56±0.46	1.88±0.90	<0.01
Change from baseline	-0.03±0.26	-0.01±0.28	NS
Change from baseline, P-value†	NS	NS	
Mean luminal diameter (mm)			
Baseline	2.22±0.63	2.58±0.90	<0.01
4 months	2.20±0.65	2.55±0.90	<0.01
Change from baseline	-0.02±0.20	-0.03±0.28	NS
Change from baseline, P-value†	NS	NS	
Diameter stenosis (%)			
Baseline	34±14	30±20	NS
4 months	36±16	31±21	NS
Change from baseline	+2±14	+1±11	NS
Change from baseline, P-value†	NS	NS	
Atheromatosis severity score			
Baseline	6.36±2.04	4.21±2.26	<0.01
4 months	6.38±2.03	4.22±2.26	<0.01
Change from baseline	+0.02±1.83	+0.01±1.44	NS
Change from baseline, P-value†	NS	NS	

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

In order to provide more insight in changes of individual coronary segments, a categorical comparison of the absolute change in percentage diameter stenosis was made between coronary segments located within the injected territory and segments located outside the injected territory. As shown in **Table 4**, the change in percentage diameter stenosis was similar in both groups. Of interest, the 8 coronary segments located within the injected territory with a 10-20% increase in diameter stenosis all had a baseline diameter stenosis <50%.

Table 4. Absolute change in diameter stenosis (%)

	Segments within injected territory (n=97)		Segments outside injected territory (n=270)		P-value*
-20 % to -10 %	14	(14 %)	35	(13 %)	
-10 % to 0 %	17	(18 %)	57	(21 %)	
0 % to +10 %	58	(60 %)	151	(56 %)	NS
+10 % to +20 %	8	(8 %)	25	(9 %)	
+20 % to +30 %	0	(0 %)	2	(<1 %)	

*Assessed by Chi-square analysis

Table 5 Arterial and venous bypass grafts

A. Arterial bypass grafts (n=28)

Variable	Baseline	4 months	P-value*
Segment length (mm)	110±30	109±31	NS
Reference diameter (mm)	3.30±0.45	3.31±0.45	NS
Minimal luminal diameter (mm)	2.79±0.34	2.79±0.33	NS
Mean luminal diameter (mm)	3.42±0.37	3.41±0.39	NS
Diameter stenosis (%)	15±5	16±6	NS
Atheromatosis severity score	1.50±1.52	1.51±1.50	NS

*Assessed by paired Student's t-test

B. Venous bypass grafts (n=26)

Variable	Baseline	4 months	P-value*
Segment length (mm)	83±93	84±95	NS
Reference diameter (mm)	3.84±0.91	3.84±0.93	NS
Minimal luminal diameter (mm)	2.78±0.72	2.77±0.73	NS
Mean luminal diameter (mm)	3.93±1.00	3.91±1.00	NS
Diameter stenosis (%)	27±10	27±12	NS
Atheromatosis severity score	3.94±2.64	3.95±2.62	NS

*Assessed by paired Student's t-test

Angiographic Follow-up: Bypass Grafts

In the 28 patients with a history of coronary artery bypass grafting, a total of 28 arterial and 26 venous bypass grafts were patent at the time of cell injection. During the 4 months follow period, no significant change in reference diameter, minimal luminal diameter and mean luminal diameter was noted in arterial or in venous bypass grafts (**Table 5**). Similarly, the percentage diameter stenosis and the atheroma severity score remained unchanged.

Discussion

The main finding from the current study is that there was no evidence of progression of coronary atherosclerosis following intramyocardial bone marrow cell transplantation in patients with chronic myocardial ischemia. In particular, there were no adverse coronary events during the 12 months follow-up period and quantitative coronary angiography did not demonstrate a decrease in luminal diameter or an increase in percentage diameter stenosis. Furthermore, a reduction in anginal symptoms with an increased left ventricular ejection fraction and an improved myocardial perfusion was observed.

Until now, the effect of cardiac bone marrow cell transplantation on the progression of atherosclerosis has not systematically been evaluated in patients with chronic ischemic heart disease. To date, several clinical studies investigated the safety and potential efficacy of bone marrow cell injection for chronic ischemia.[14,25-27] Although routine angiographic follow-up was not performed, the studies reported that intramyocardial bone marrow cell injection was safe. However, 7 of 27 patients in the study by Fuchs et al. required a coronary intervention within 12 months after cell transfer because of late in-stent restenosis or aggravation of coronary atherosclerosis.[25] Although the majority of revascularization procedures were performed in coronary artery segments supplying non-injected territories, it cannot be excluded that cell injection contributed to accelerated progression of coronary atherosclerosis since a control group was not included and routine repeat coronary angiography was not performed in the entire study population.

Until now, angiographic follow-up after cardiac cell transplantation has only been performed in a few studies in patients receiving intracoronary cell infusion after percutaneous coronary intervention for acute myocardial infarction. The different studies have, however, yielded conflicting results. The MAGIC study reported an unexpected high rate of restenosis after intracoronary infusion of G-CSF mobilized cells in post-infarction patients.[13] In addition, there was a close correlation between gain of neointimal volume and improvements of systolic function, suggesting that cell therapy accelerated neointimal growth in proportion to the improvement in left ventricular contractility.[13] In agreement with the results from the MAGIC study, Bartunek et al. reported that patients treated with intracoronary infusion of CD133+ enriched bone marrow cells showed a higher incidence of coronary events as compared to control patients.[8,28] Moreover, accelerated progression of atherosclerosis was observed in coronary segments downstream from the site of cell transplantation.[8,28] Other studies in patients with acute myocardial infarction however, did not reveal a relationship between bone marrow cell transplantation and increased rate of restenosis or accelerated atherosclerosis.[7,29-31] For example, quantitative coronary angiography at 6 months follow-up in the BOOST trial showed no difference between the bone marrow cell group and the control group with respect to the incidence of in-stent restenosis.[29] Similarly, the TOPCARE-AMI investigators reported that intracoronary cell infusion did not aggravate restenosis development or progression of atherosclerosis within the infused coronary artery.[30]

The conflicting reports regarding the potential for accelerated atherosclerosis after bone marrow cell transplantation for acute myocardial infarction have raised discussion regarding the underlying mechanism. According to the MAGIC investigators, possible explanations for aggravation of atherosclerosis include differentiation of G-CSF mobilized cells into neointimal smooth muscle cells, induction of angiogenesis within atherosclerotic lesions, and aggregation of mobilized inflammatory cells within the plaque.[13] On the other hand, Bartunek et al. suggested that repetitive balloon inflations at the time of cell infusion may have caused endothelial damage, with subsequent development of in-stent restenosis.[8] In addition, secretion of pro-inflammatory cytokines by the therapeutic cells may have enhanced progression of atherosclerosis in the distal coronary segments.[28] However, since aggravation of atherosclerosis was not observed in all studies, the TOPCARE-AMI investigators speculated that bone marrow-cell independent factors were responsible for the progression of atherosclerosis in the 2 above-described studies.[30] For example, G-CSF pre-treatment of the infarct-related artery in the MAGIC study may have contributed to the progression of atherosclerosis as G-CSF exhibits pro-inflammatory properties. In addition, the TOPCARE-AMI investigators suggested that the aggravation of atherosclerosis described by Bartunek et al. could have been related to the murine antibody that was used to isolate the CD133+ cell fraction. This murine antibody (which was not removed from the cells before cell transplantation) may have caused an inflammatory reaction in the coronary segments downstream from the site of cell transplantation. The results from the current study are in line with the findings from the BOOST and TOPCARE-AMI study, but extend the observations to patients with advanced coronary artery disease and chronic myocardial ischemia. In addition, the absence of progression of atherosclerosis in the current study provides additional evidence that cardiac bone marrow cell transplantation itself is not associated with aggravation of atherosclerosis.

Several limitations of the current study have to be addressed. First, the exclusion of coronary segments that were opacified solely by collateral circulation may have biased the results. In particular, this may have resulted in a relatively mild diameter stenosis percentage, which may not totally reflect the severity of coronary artery disease. Another limitation of this prospective, observational study (which was primarily designed to assess the safety of intramyocardial bone marrow cell transplantation in patients with chronic ischemia) is the lack of a control group. As a consequence, the described reduction in anginal symptoms, the increase in left ventricular ejection fraction and the improvement in myocardial perfusion cannot unambiguously be attributed to bone marrow cell transplantation. Future randomized, placebo-controlled studies are warranted to further investigate the beneficial effects of bone marrow cell transplantation in patients with chronic myocardial ischemia.

Conclusion

The findings of the present study do not support a significant progression of atherosclerosis following bone marrow cell transplantation in patients with chronic myocardial ischemia. Moreover, bone marrow cell transplantation appears to reduce anginal symptoms, increase left ventricular contractility and improve myocardial perfusion. The absence of progression of atherosclerosis and the favorable effects on anginal symptoms, left ventricular function and myocardial perfusion, justify the performance of future randomized placebo-controlled studies to assess the efficacy of bone marrow cell transplantation for chronic myocardial ischemia.

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