Cardiac bone marrow cell injection for chronic ischemic heart disease

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Chapter 4

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Electrophysiological and Arrhythmogenic Effects of Intramyocardial Bone Marrow Cell Injection in Patients with Chronic Ischemic Heart Disease

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Abstract

Introduction: Bone marrow cell injection has been introduced to treat patients with ischemic heart disease. However, focal application of bone marrow cells may generate an arrhythmogenic substrate. The aim of the current study was to assess the electrophysiological and arrhythmogenic effects of intramyocardial bone marrow cell injection in patients with chronic myocardial ischemia.

Methods: Bone marrow was aspirated in 20 patients (65±11 years, 19 male) with drug-refractory angina and myocardial ischemia. Electroanatomical mapping (NOGA, Biosense-Webster, Waterloo, Belgium) was performed during mononuclear cell isolation. Areas for cell injection were selected based on the localization of ischemia on SPECT. These areas were mapped in detail to evaluate local bipolar electrogram duration, amplitude and fragmentation. Mononuclear cells were injected in the ischemic area with the NOGA system. SPECT and electroanatomical mapping were repeated at 3 months. Holter monitoring was repeated at 3 and 6 months.

Results: SPECT revealed a decrease in the number of segments with ischemia (3.5±2.5 vs. 1.1±1.0 at 3 months; P<0.01) and an increased left ventricular ejection fraction (44±13% vs. 49±17% at 3 months; P=0.02). The number of ventricular premature beats remained unchanged (10±24x10^2/24h vs. 8±23x10^2/24h at 3 months (P=NS) and 12±30x10^2/24hr at 6 months (P=NS)). At 3 months follow-up, bone marrow cell injection did not prolong electrogram duration (15.9±4.6 ms vs. 15.6±4.0 ms; P=NS), decrease electrogram amplitude (3.8±1.5 mV vs. 3.8±1.5 mV; P=NS), or increase fragmentation (2.0±0.5 vs. 1.9±0.4; P=NS).

Conclusion: Intramyocardial bone marrow cell injection does not increase the incidence of ventricular arrhythmia and does not alter the electrophysiological properties of the injected myocardium.

Introduction

Intramyocardial cell transplantation is currently being investigated as a potential therapy for chronic ischemic heart disease.[1] Through application of therapeutic cells to ischemically-damaged myocardium, this novel treatment modality aims to enhance left ventricular function and improve myocardial perfusion. At present several cell types including bone marrow-derived cells and skeletal myoblasts have been used in clinical studies for this purpose.[2-8] The increased incidence of ventricular tachycardia in clinical studies using human skeletal myoblast transplantation highlighted the importance of a systematic assessment of the potential arrhythmogenic consequences of cell therapy.

A few pre-clinical studies addressed the electrophysiological characteristics of bone marrow cells. From these studies it appears that focal application of bone marrow cells can potentially generate an arrhythmogenic substrate. For example, Chang et al. recently reported that mixtures of bone marrow-derived mesenchymal stem cells and neonatal rat cardiomyocytes resulted in an arrhythmogenic substrate that is characterized by decreased conduction velocity and easily inducible sustained reentrant arrhythmia.[9] Similarly, we previously demonstrated that transmission of the electrical impulse across human adult bone marrow-derived mesenchymal stem cells is characterized by slow conduction, reduced depolarization rates and low-amplitude electrical activity decaying with distance.[10]

In the clinical setting 5 studies evaluated the safety and feasibility of intramyocardial bone marrow cell injection for chronic myocardial ischemia.[2-6] Holter monitoring in these studies demonstrated no increase in the incidence of ventricular arrhythmia. However, no study thus far systematically evaluated the electrophysiological effects of intramyocardial bone marrow cell injection in patients with chronic myocardial ischemia.

Three-dimensional electroanatomical catheter mapping could potentially contribute in the evaluation of the electrophysiological effects of bone marrow cell injection. Based on the analysis of bipolar electrograms, this technique allows accurate identification of zones of slow conduction and characterization of an arrhythmogenic substrate.[11-13]

The present study investigated the electrophysiological and arrhythmogenic effects of intramyocardial bone marrow cell injection in patients with chronic myocardial ischemia and subsequent drug-refractory angina. Electroanatomical mapping of the injected ischemic myocardial region was performed to assess changes in local bipolar electrogram characteristics and 24-hour Holter monitoring was used to evaluate the incidence of ventricular arrhythmia.

Methods

Patients

Patients with severe angina pectoris (Canadian Cardiovascular Society (CCS) class III or IV) despite optimal medical therapy were included in the current study if stress-rest technetium-99m tetrofosmin single photon emission computed tomography (SPECT) revealed the presence of stress-inducible ischemia. All patients were ineligible for percutaneous or surgical revascularization as assessed by coronary angiogram. The exclusion criteria were: acute myocardial infarction within 6 months of enrollment, history of malignancy, renal dysfunction (serum creatinine >200 µmol/L) and unexplained hematological or biochemical laboratory abnormalities. The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from all patients.

Study Protocol

Baseline evaluation included assessment of the patients' clinical status according to the CCS classification and 24-hour Holter monitoring to document heart rhythm, heart rate, ventricular premature beats and the occurrence of (non-) sustained ventricular tachycardia. In order to identify the location of stress-inducible myocardial ischemia and assess left ventricular ejection fraction, stress-rest technetium-99m tetrofosmin gated SPECT was performed.

Immediately before bone marrow cell injection, 3-dimensional electroanatomical mapping of the left ventricle was performed using the NOGA system (Biosense-Webster, Waterloo, Belgium). The electroanatomical map was used to evaluate the local bipolar electrogram characteristics of the myocardial region with ischemia and guide the bone marrow cell injections. Immediately after the procedure, 2-day continuous heart rhythm monitoring was started.

Follow-up evaluations performed 3 and 6 months after the injection procedure consisted of a clinical assessment (CCS classification) and 24-hour Holter recording to monitor ventricular arrhythmia. At 3 months follow-up, electroanatomical mapping was repeated to reassess the local electrophysiological characteristics of the injected myocardium. In addition, stress-rest gated SPECT was repeated at 3 months follow-up to reassess myocardial ischemia and left ventricular ejection faction. Cardiovascular medication remained unchanged during the 6 months follow-up period.

Electroanatomical Mapping and Bone Marrow Cell Injection

At the day 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 mapping with the NOGA system, which has been described in detail previously.[14,15] All patients received an intravenous dose of 7,500 U heparin.

After left ventricular angiography, left ventricular endocardial mapping was performed via femoral artery access and a retrograde aortic approach using a 7-French NOGAStar catheter (2 mm tip, 2 electrodes, interelectrode distance 0.5 mm; Biosense-Webster). The high-pass filters were set at 30 Hz and the low-pass filters at 500 Hz.

Landmark points outlining the endocardial left ventricular boundaries (apex, aortic outflow, mitral inflow) were acquired under fluoroscopic guidance. The catheter was dragged over the endocardial surface to record electrograms at different sites and to simultaneously determine the shape and volume of the left ventricle. Points were only acquired when the catheter tip had a stable position (using local stability, loop stability and cycle length stability as specified by the manufacturer)[14,15] perpendicular to the myocardial wall. In order to provide high spatial resolution and minimize interpolation between actual data points by the system, the filling threshold was set at 15 mm. This setting allows the NOGA system to fill triangles between acquired points using an interpolation algorithm, so that distances between points larger than 15 mm away from each other are left blank until points in between are sampled. After completion of the map, the mapping points of the internal sites of the left ventricle (distance from fitting plane >35 mm) and mapping points with unsatisfactory stability were removed.

Based on the localization of ischemia on Tc-99m tetrofosmin SPECT, the region of interest (= myocardial area with ischemia on SPECT at which cell injections were targeted) was delineated on the NOGA map. Therefore, by definition the region of interest comprised ischemic but viable (unipolar voltage ≥6.9mV, bipolar voltage ≥1.5 mV)[13,16,17] myocardium.

For further evaluation of the local electrophysiological characteristics of the region of interest, 25-30 additional points were acquired in this region. Accordingly, a high-density map of the region of interest was derived. In order to allow post-procedural off-line analysis, the additional electrograms recorded in the region of interest were simultaneously stored on the NOGA system and on an electrophysiological recording (EP) system (Boston Scientific, Natick, MA, USA).

Immediately after completion of the high-density map of the region of interest the mapping catheter was replaced by the MyoStar injection catheter (Biosense-Webster), from which a 27-gauge injection needle can be advanced by 4-6 mm for direct intramyocardial injection. The injection catheter was directed towards the region of interest. Before each injection of cells in the myocardium, the following criteria had to be met: perpendicular position of the catheter to the myocardial wall, excellent loop stability (<4 mm), unipolar voltage ≥6.9 mV and the presence of a premature ventricular contraction upon extending the needle into the myocardium. Thereafter, 8-13 injections of approximately 0.2 ml cell suspension each were delivered.

Repeat Electroanatomical Mapping

Repeat electroanatomical mapping at 3 months follow-up was performed in an identical way as the mapping procedure prior to bone marrow cell injection. In brief, a NOGA map of the entire left ventricle (fill threshold 15 mm) was made with the NOGAStar catheter. Then, the region of interest (= the myocardial region with ischemia at baseline in which the bone marrow cells were injected) was delineated on the NOGA map. The correct localization of the region of interest was verified by comparing the localization on the baseline bulls eye reconstruction to the 3 months bulls eye reconstruction. Thereafter, a high density map of the region of interest was made by acquiring 25-30 points in this region. The additional electrograms acquired from the region of interest were stored on the EP system for post-procedural offline analysis.

Postprocedural Electrogram Analysis

An experienced observer who was blinded to all clinical data analyzed the electrograms acquired in the region of interest which were transferred to the EP system. Of note, these bipolar electrograms were all recorded with the NOGAStar catheter and displayed with fixed gain (1 mV/cm) at 200 mm/s.

Signal duration was defined as the distance between the earliest sharp peak deflection and the latest sharp peak deflection. Peak-to-peak amplitude was measured automatically by the EP system and adjusted manually if necessary. Fragmentation was defined as a multicomponent (>2 deflections) signal.[18,19] The degree of fragmentation was quantified by the number of positive electrogram deflections. As reported previously, cut-off values for distinguishing normal electrograms were ≤40 ms for duration,[11] ≥1.5 mV for amplitude,[13,17] and ≤4 positive peak deflections for fragmentation.[11] Examples of measurement of electrogram duration, amplitude and fragmentation are shown in **Figure 1**.

SPECT Imaging

For SPECT imaging, a previously described 2-day stress-rest protocol was used.[5] For stress imaging, pharmacological stress (intravenous administration of adenosine 0.14 mg/kg/min for 6 minutes (n=18), 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, ECG-gated 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)[20] with the use of quantitative gated SPECT (QGS)-software (Cedars-Sinai Medical Center, Los Angeles, California, USA).[21] When tracer uptake at stress was ≥75% of maximum tracer activity, segments were considered to be normally perfused. Segments with <75% tracer uptake

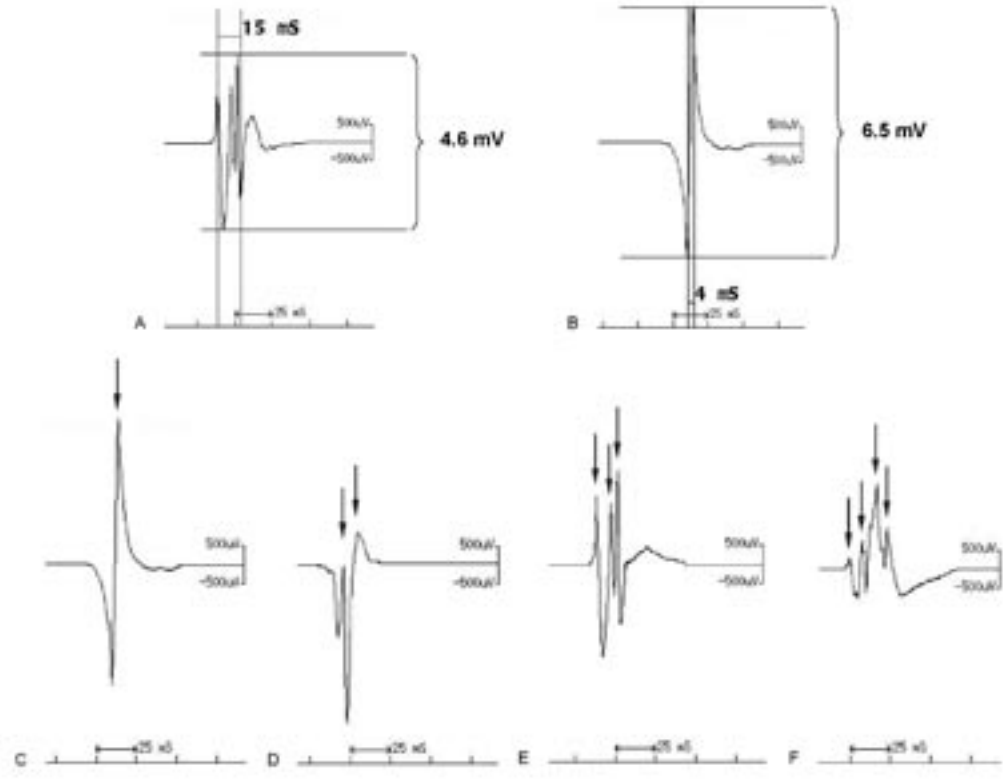


Figure 1
Measurement of duration, amplitude and fragmentation from bipolar electrogram recordings. A-B: Measurement of electrogram duration and electrogram amplitude. Electrogram duration was defined as the distance between the earliest sharp peak deflection and the latest sharp peak deflection. C-F: Measurement of electrogram fragmentation. The degree of fragmentation was defined as the number of positive electrogram deflections recorded at a gain of 1 mV/cm at 200 mm/sec.

at stress in which tracer uptake increased >10% at rest were considered to be ischemic. Left ventricular volumes and ejection fraction were calculated using QGS software.[22]

Statistical Analysis

Data were expressed as mean±SD. Comparison of continuous data was performed using the paired, Student's t test. Proportions were compared using Chi-square analysis. A P-value <0.05 was considered statistically significant.

Results

The baseline characteristics of the 20 patients (mean age 65±11 years, 19 male) receiving intramyocardial bone marrow cell injection are summarized in **Table 1**. By definition, all patients had severe angina and 12 patients were in CCS class III and 8 in CCS class IV.

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

Characteristics		
Age (years)	65±11	
Gender (male)	19	(95%)
Cardiovascular history		
Prior myocardial infarction	15	(75%)
Prior percutaneous coronary intervention	14	(70%)
Prior coronary artery bypass grafting	18	(90%)
Prior implantable cardioverter-defibrillator	3	(15%)
Left ventricular ejection fraction (%)	44±13	
Canadian Cardiovascular Society class	3.4±0.5	

In addition, 4 patients had severe heart failure (New York Heart Association class III or VI). Mean left ventricular ejection fraction was 44±13% (range 18-64%). Three patients (with a left ventricular ejection fraction <35% and a previous myocardial infarction) had an implantable cardioverter-defibrillator (ICD). Patients' medications included long-acting nitrates (20/20), β-blockers (18/20) and calcium channel blockers (16/20). Medication remained unchanged during the study period.

Electroanatomical Mapping

The mean procedural time to acquire a complete electroanatomical map of the left ventricle was 31±10 min before cell injection and 29±7 min at 3 months (P=NS). During the baseline mapping procedure actual electrograms were recorded from 92±18 sites, whereas during the follow-up mapping procedure actual electrograms were recorded from 91±20 sites (P=NS). After careful filtering of the internal points (distance from fitting plane >35 mm) and mapping points with unsatisfactory stability, the mean number of points drawing the left ventricular silhouette was 82±19 before cell injection and 80±17 at 3 months follow-up (P=NS).

The region of interest (which was delineated on the NOGA map on the basis of the localization of ischemia on SPECT) was located anterior in 4 patients, lateral in 3 patients, inferior in 7 patients and septal in 6 patients. The region of interest comprised 15±4% of the size of the entire left ventricle. At baseline and at 3 months follow-up a similar number of points was acquired in the region of interest (34±7 vs. 31±7; P=NS).

Bone Marrow Cell Injection

The final preparation of injected cells contained 97±9x10^6 mononuclear cells. Cell viability was 98±1% and the CD34+ cell fraction was 2.4±1.3%. Patients received 10.1±1.6 injections of approximately 0.2 ml each. The time for cell injection was 25±10 min. No periprocedural complications occurred. Sustained ventricular tachycardia did not occur

during mapping or cell injection. In addition, 2-day continuous heart rhythm monitoring did not reveal sustained ventricular tachycardia. Patients were discharged 2.5±0.8 days after the injection procedure.

Clinical Follow-up

At 6 months follow-up, no patient died or was lost to follow-up. None of the patients experienced an acute myocardial infarction or underwent a (surgical or percutaneous) revascularization procedure. At 3 months follow-up 1 patient was hospitalized for syncope because of bradycardia. Continuous heart rhythm monitoring (7 days) in this patient reveled several episodes of sinus bradycardia (35/min), but no ventricular arrhythmia. At 6 months, 1 patient with an ICD received an appropriate shock because of sustained ventricular tachycardia (CL 180 ms). Of interest, in the 6 months prior to bone marrow cell injection this patient had received 2 appropriate shocks because of sustained ventricular tachycardia (1st episode CL 190 ms; 2nd episode CL 170 ms). Device interrogation in the other 2 ICD patients revealed no episodes of sustained ventricular tachycardia.

Mean CCS class improved from 3.4±0.5 at baseline to 2.5±0.6 at 3 months (P<0.01) and remained unchanged at 6 months follow-up (2.7±0.7; P<0.01 vs. baseline and P=NS vs. 3 months). The number of ischemic segments per patient decreased from 3.5±2.5 at baseline to 1.1±1.0 at 3 months (P<0.01). An example of resolution of lateral ischemia is shown in **Figure 2**. Left ventricular ejection fraction increased from 44±13% at baseline to 49±17% at 3 months (P=0.02). There was a trend toward a reduction in end-systolic volume (94±68 ml vs. 89±69 ml; P=0.05) whereas end-diastolic volume remained unchanged (153±79 ml vs. 154±78 ml; P=NS).

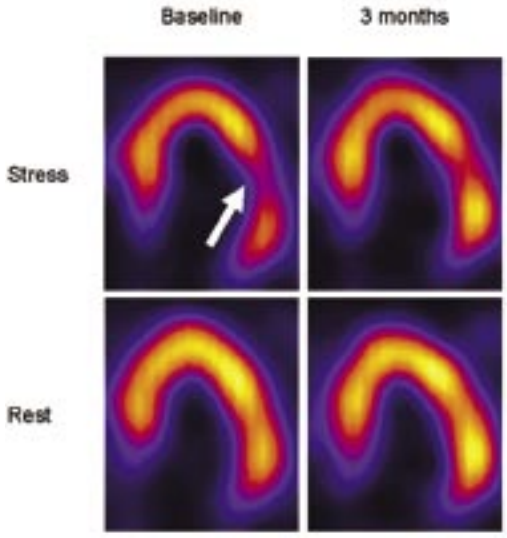


Figure 2
Stress and rest SPECT images (horizontal long axis) at baseline and at 3 months follow-up from a representative patient showing relief of stress-inducible ischemia in the lateral wall (arrow).

Table 2. Twenty-four-hour Holter monitoring

	Baseline	3 months	6 months
Rhythm (SR/Afib/ PMR)	15/2/3	16/1/3	16/1/3
Heart rate average	66±13	65±12	65±12
Heart rate minimum	45±9	46±8	47±7
Heart rate maximum	104±25	103±25	100±21
Total VPB (x10^2 beats/24h)	10±24	8±23	12±30
Isolated (x10^2/24h)	10±22	8±23	11±29
Couplets/24h	31±10	24±11	26±9
Salvos/24h	1±3	1±1	1±2
No. patients with NSVT	2/20	2/20	2/20

Afib = atrial fibrillation; NSVT = non-sustained ventricular tachycardia; PMR = pacemaker rhythm; PVB = ventricular premature beats; SR = sinus rhythm
P=NS for all comparisons vs. baseline

Holter Monitoring

Twenty-four-hour Holter monitoring was performed at baseline, at 3 and at 6 months follow-up. As shown in **Table 2**, bone marrow cell injection did not alter heart rhythm or heart rate. In addition, the total number of ventricular premature beats per 24 hour remained unchanged (10±24x10^2/24h at baseline vs. 8±23x10^2/24h at 3 months (P=NS) vs. 12±30x10^2/24h at 6 months (P=NS vs. baseline). Non-sustained ventricular tachycardia was recorded in 2/20 patients at baseline. In the same 2 patients, non-sustained ventricular tachycardia was recorded at 3 and at 6 months. The duration, cycle length and morphology of the non-sustained ventricular tachycardia recorded at baseline and during follow-up

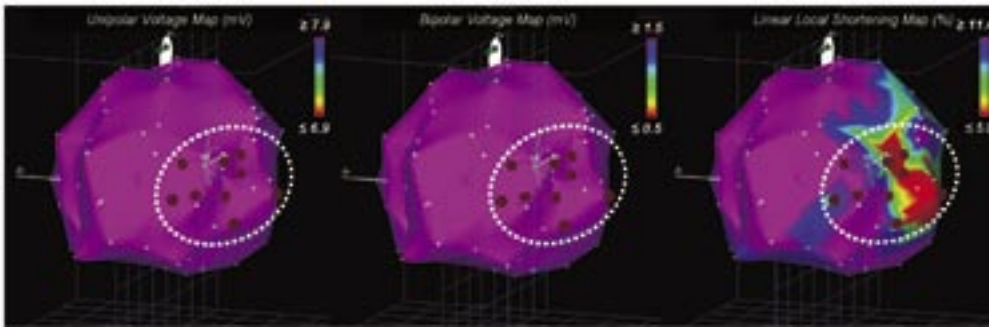
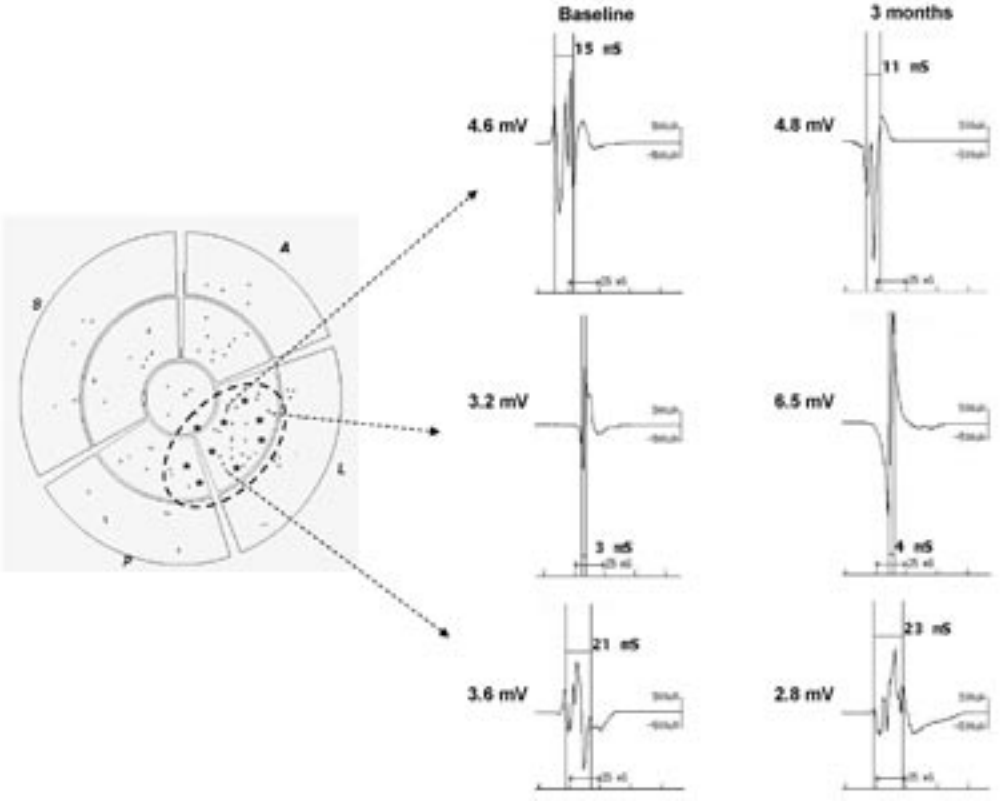


Figure 3
A: Color-coded NOGA maps viewed from a left anterior oblique position from the same patient as in Figure 2 at baseline. Based on the localization of ischemia (lateral), the region of interest was delineated on the NOGA map (dotted line). In the region of interest, the NOGA maps show normal (unipolar (left) and bipolar (middle)) voltages, whereas linear local shortening in this region is diminished (right). The dark-red dots indicate the 9 injection sites.



B: NOGA polar map from the same patient (A = anterior, L = lateral, P = posterior, S = septal). Black asterisks indicate the 9 injection sites. Bipolar electrograms recorded from the ischemic (but viable) myocardium (= region of interest) at baseline (left column) show normal electrogram duration (≤40 ms), normal amplitude (≥1.5 mV) and normal fragmentation (≤4). Similarly, bipolar electrograms recorded in the same region at 3 months follow-up (right column) show normal electrogram duration, amplitude and fragmentation.

were almost identical (1st patient: duration 4-8 beats, CL 360-390 ms, intermediate axis; 2nd patient: duration 5-10 beats, CL 330-375 ms, right axis). Sustained ventricular tachycardia was not documented on Holter recordings at baseline or at follow-up.

Electrogram Characteristics

An example of the way the region of interest was delineated on the NOGA map in a patient with lateral ischemia (same patient as in **Figure 2**) is shown in **Figure 3A**. **Figure 3B** displays examples of electrograms recorded in the region of interest at baseline and at 3 months follow-up.

Before cell injection, the electrograms recorded in the region of interest showed normal amplitude, normal duration and normal fragmentation. **Figure 4** summarizes the characteristics of the bipolar electrograms which were registered in the region of interest

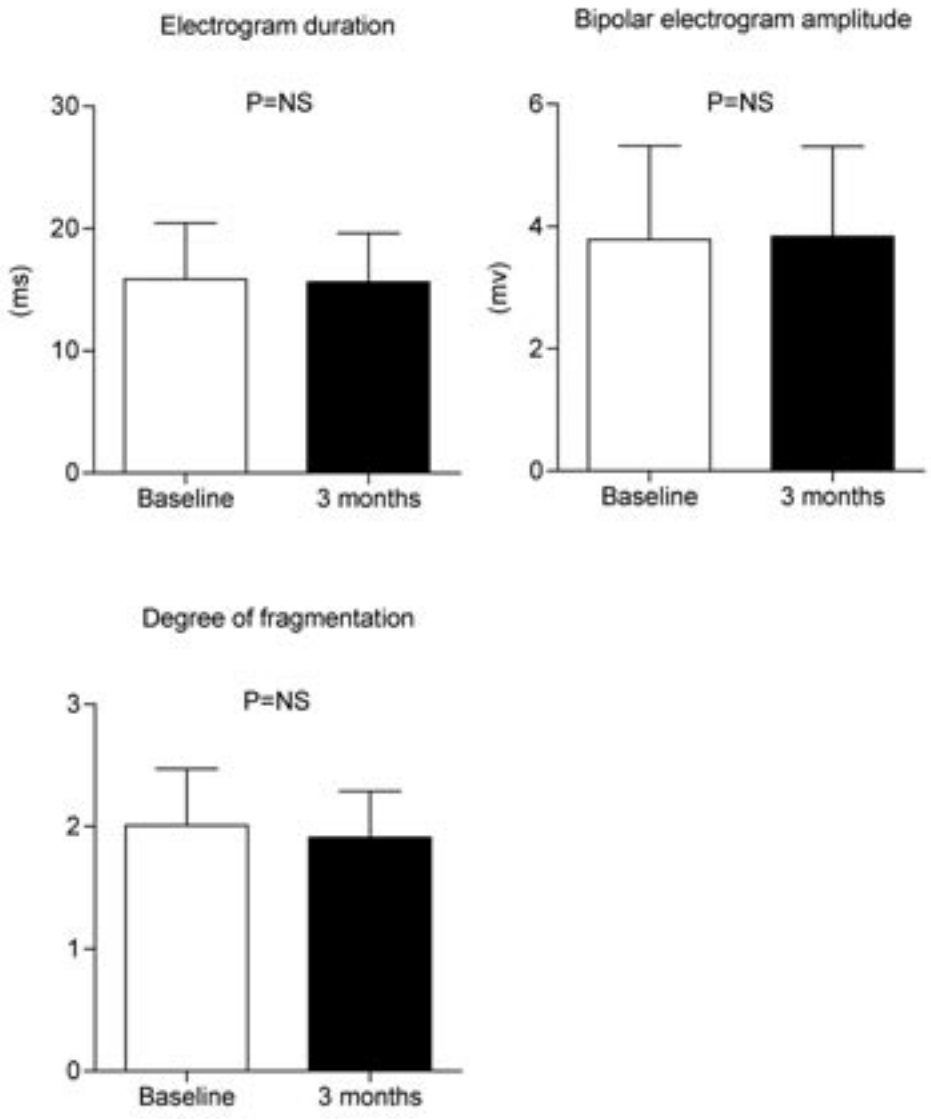


Figure 4
Electrogram duration (upper left panel), electrogram amplitude (upper right panel) and the degree of fragmentation (lower panel) in the region of interest at baseline and at 3 months follow-up as assessed by electroanatomical mapping.

at baseline and at 3 months follow-up. Bone marrow cell injection did not prolong electrogram duration (15.9±4.6 ms at baseline vs. 15.6±4.0 ms at 3 months; P=NS), decrease electrogram amplitude (3.8±1.5 mV vs. 3.8±1.5 mV; P=NS), or increase the degree of fragmentation (2.0±0.5 at baseline vs. 1.9±0.4 at 3 months; P=NS).

Discussion

This is the first study to evaluate the electrophysiological effects of intramyocardial bone marrow cell injection in patients with chronic myocardial ischemia and subsequent angina pectoris. The main finding from our study is that intramyocardial bone marrow cell injection is not arrhythmogenic and does not alter the electrophysiological properties of the injected myocardium. Furthermore, major clinical improvements were observed: anginal symptoms reduced, myocardial perfusion improved and left ventricular ejection fraction increased.

Previous in Vitro Model Studies

Bone marrow cell transplantation is emerging as a novel therapeutic option for patients with chronic ischemic heart disease. However, to date controversy exists whether this new treatment modality increases the vulnerability for arrhythmia. In vitro model studies have suggested that increased electrophysiological heterogeneity after bone marrow cell transplantation could increase the incidence of arrhythmia. In an in vitro study, we previously investigated the electrophysiological properties of human adult bone marrow mesenchymal stem cells.[10] Despite the presence of functional connexin-43 positive gap junctions between bone marrow cells and neighboring cardiomyocytes, conduction velocity across bone marrow cells was approximately 11-fold slower than across cardiomyocytes. Intra- and extra-cellular electrogram recordings indicated that impulse transmission across bone marrow cells was characterized by slow conduction, reduced depolarization rates and low-amplitude electrical activity. Similarly, Chang et al., performing optical mapping of a model of cardiac bone marrow cell transplantation, reported that conduction velocity was significantly depressed in cocultures containing >10% bone marrow mesenchymal stem cells.[9] Sustained reentrant arrhythmia (the in vitro equivalent of sustained monomorphic ventricular tachycardia) was easily inducible by rapid pacing in cocultures containing at least 10% bone marrow cells, but not in cocultures containing 1% bone marrow cells. Of note, triggered activity and increased automaticity were not observed to be the underlying mechanism of the arrhythmia. As with intramyocardial injection large numbers of cells are directly introduced in the myocardium (resulting in an uneven distribution of the cells), Chang et al. expected this application mode to be more arrhythmogenic than intravascular infusion.[9] Furthermore, local tissue injury or scar formation after intramyocardial injection could increase tissue heterogeneity and therefore increase the risk for arrhythmia.

Previous in Vivo Model Studies

The results from in vivo model studies support the hypothesis that bone marrow cell injection induces electrophysiological tissue heterogeneity. Chen et al. described that autologous bone marrow mesenchymal stem cell transplantation prolonged local

activation time and increased the activation time dispersion in a rabbit heart failure model.[23] Furthermore, there was a positive correlation between activation time dispersion and the number of bone marrow cells in the pacing site. Nevertheless, programmed stimulation could not induce ventricular tachycardia or ventricular fibrillation. Another group demonstrated in a swine myocardial infarction model that transplantation of bone marrow-derived mesenchymal stem cells significantly shortened epicardial effective refractory periods.[24] The same group also reported that intramyocardial injection of bone marrow mesenchymal stem cells resulted in overexpression of cardiac tenascin, increased cardiac nerve sprouting and increased sympathetic hyperinnervation.[25] Sympathetic nerve activation exerts significant effects on electrophysiological properties such as refractoriness and conduction velocity of myocardial cells.[26,27] Increased and heterogeneous cardiac innervation might facilitate the initiation of ventricular arrhythmia. However, increased occurrence of ventricular arrhythmia or sudden death in bone marrow-treated animals was not reported.

Previous Clinical Studies

In the clinical setting, 5 studies have evaluated the safety and feasibility of intramyocardial bone marrow cell injection for chronic myocardial ischemia.[2-6] Based on Holter monitoring in these studies it appears that bone marrow cell injection does not increase the incidence of ventricular arrhythmia. However, in the study by Perin et al., 1 patient in the treatment group died 14 weeks after the injection procedure, presumably of sudden cardiac death.[28]

Electrophysiological mapping or programmed ventricular stimulation was until now not performed in patients with chronic myocardial ischemia. Only 1 randomized study in patients receiving intracoronary bone marrow cell infusion after acute myocardial infarction evaluated the inducibility of ventricular arrhythmia.[29] Programmed ventricular stimulation at 6 months revealed non-sustained ventricular tachycardia in 1 control patient and in 1 bone marrow cell transfer patient. Ventricular fibrillation was inducible in 1 control patient. Wollert et al. concluded that there was no evidence for an arrhythmogenic effect of intracoronary bone marrow cell transplantation.

Interpretation of Electrophysiological Findings in the Current Study

The majority of the pre-clinical studies support electrical heterogeneity and local conduction delay as the underlying mechanism of the potential arrhythmogenic effect of bone marrow cell injection. In addition, intramyocardial injections in viable myocardium may result in scar formation which may aggravate local conduction delay and facilitate reentry. The correlation between bipolar electrogram abnormalities after myocardial infarction and the underlying arrhythmogenic substrate has been studied extensively.[30-32] Bipolar electrogram amplitude was related to the number of viable, activated muscle fibers, whereas electrogram duration and fragmentation were related to local conduction

properties. In addition, the extent of the area with abnormal electrograms appeared to correlate with the likelihood to induce ventricular tachycardia.[33]

The absence of alterations in electrogram amplitude in the current study (using a high density of acquired points in the region of interest) makes significant scar formation in the injected myocardium unlikely. In addition, the lack of increase in signal fragmentation and duration supports unchanged local conduction properties. The use of a 3-dimensional electroanatomical mapping system in combination with anatomical landmarks provides reproducible delineation of the injected area.

The results of the current study are in line with the study in acute myocardial infarction patients, but extend the observations to patients receiving intramyocardial bone marrow cell injection for chronic ischemia. Accordingly, it can be concluded that focal bone marrow cell injection does not generate clinically-detectable electrophysiological tissue heterogeneity. In addition, potential increased cardiac nerve sprouting, increased sympathetic hyperinnervation and local tissue injury did result in clinically-detectable electrophysiological alterations of the injected myocardium.

Clinical Improvement

Patients in the present study exhibited a significant improvement in anginal symptoms that was accompanied by improved myocardial perfusion and left ventricular ejection fraction. The magnitude of clinical improvement observed in the current study is in line with previous safety/feasibility studies of bone marrow cell transplantation in patients with chronic myocardial ischemia and subsequent angina pectoris.[2-5] Since cell injection did not alter local electrogram characteristics it is unlikely that the injected cells differentiated into substantial amounts of cardiomyocytes. Most probably, the mechanism underlying the clinical improvement following bone marrow cell injection is related to promotion of angiogenesis, as proposed in animal model studies. For example, Fuchs et al. described that NOGA-guided bone marrow cell injection in pigs with chronic ischemia enhanced collateral flow through secretion of potent pro-angiogenic cytokines and proliferation of bone marrow-derived vascular and endothelial cells.[34]

Since the current study comprised no control group, the clinical improvement cannot be unambiguously attributed to bone marrow cell injection. Other contributing factors could be placebo effect, spontaneous improvement and medical therapy. However, it is rather uncommon that myocardial perfusion and left ventricular function improve without (coronary) intervention, and cardiovascular medication remained unchanged in all patients during the entire study period.

Study Limitations

First, measurement of electrogram duration by electrophysiological mapping is influenced by the direction of the wavefront in relation to the bipole. Second, programmed ventricular stimulation might be of additional value for the evaluation of the inducibility of ventricular

tachycardia after bone marrow cell injection. We did not perform programmed ventricular stimulation as we expected that the inducibility of ventricular tachycardia was low (most patients had a preserved left ventricular function, no large prior myocardial infarction and no previous ventricular tachycardia). In addition, programmed ventricular stimulation would also prolong and complicate the injection procedure. Third, the follow-up period of 6 months and the lack of a control group can be considered a limitation. Future randomized placebo-controlled trials should investigate the long-term electrophysiological effects of bone marrow cell injection.

Conclusions

In summary, intramyocardial bone marrow cell transplantation in patients with chronic ischemic heart disease is safe since it does not alter the electrophysiological properties of the injected myocardium detectable with currently available mapping techniques. In addition, it does not increase the incidence of ventricular arrhythmia on repetitive Holter monitoring. Finally, bone marrow cell injection appears to reduce anginal symptoms, improve myocardial perfusion and increase left ventricular ejection fraction.

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