Original Article

Sustained Improvement in Symptoms and Exercise Capacity up to Six Months After Autologous Transendocardial Transplantation of Bone Marrow Mononuclear Cells in Patients With Severe Ischemic Heart Disease

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Objective

This study aimed at assessing the effects of autologous transendocardial transplantation of bone marrow mononuclear cells (ATTBMMC) on symptoms, exercise capacity, myocardial perfusion and contractility in patients with severe ischemic heart disease during a 6-month follow-up period.

Methods

This prospective study comprised 21 patients as follows: the first 14 patients forming the treated group, and the last 7 patients forming the control group. Initially, all patients underwent clinical and laboratory assessment, treadmill testing, echocardiography, myocardial scintigraphy, and 24-hour Holter. The bone marrow mononuclear cells (BMMC) were isolated, washed, and diluted in 0.9% saline solution for transendocardial injection in areas of viable myocardium in the treated group, (15 0.2-mL injections). All patients were reassessed in the end of 2 and 6 months of follow-up.

Results

The demographic data and other characteristics did not significantly differ between the groups in the initial evaluation. No major adverse events related to the ATTBMMC were observed. In the end of 6 months, a reduction in the ischemic area was observed on nuclear perfusion imaging (P=0.05), as was a significant improvement in symptoms, functional capacity, and left ventricular overall function.

Conclusion

This study showed that transendocardial injections of BMMC are safe in human beings with ischemic heart disease associated with severe ventricular dysfunction. The effects observed in the short run were maintained up to the sixth month of follow-up.

Key words

cells, heart failure, ischemia, revascularization, gene therapy

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Refinement of several techniques of myocardial revascularization, both percutaneous and surgical, has produced increasingly effective results regarding the reduction in morbidity and mortality due to acute and chronic heart diseases. Therefore, the number of patients with ischemic heart disease has progressively increased, accounting for more than half of all hospitalizations due to heart failure, representing more than 400,000 hospitalizations/year in Brazil according to data of the DATASUS (database of the Brazilian Public Health System) [1].

The mortality rate among patients with ischemic heart disease is high, and becomes even higher in the subgroup of patients with heart failure, exceeding the rate of 30% per year [2]. Due to their elevated morbidity and mortality, these cases consume a large amount of the health system resources.

Knowledge of the clinical characteristics of that population is fundamental to the identification and development of new techniques to fulfill the needs of those patients.

Several therapeutic strategies have been tested for the treatment of patients with disease secondary to both ventricular dysfunction and chronic perfusion deficit refractory to the available myocardial revascularization techniques. Those strategies comprise the following: intermittent or long-term use of urokinase [3-5]; neurostimulation [6]; transmyocardial revascularization by use of laser or radiofrequency, or mechanical transmyocardial revascularization [7-12]; and neoangiogenesis through implantation of endothelial growth factors [13-20]. However, none of those techniques, despite some years of development, has proved to be effective in changing the poor prognosis of those patients to justify its routine clinical use [7-12].

More recently, some alternatives related to cell therapy have begun to be developed. Several recent studies have suggested that cells originating from the bone marrow (BM) also participate intensely in the regeneration of several structures of the cardiovascular system.

Cell implantation for the treatment of cardiovascular diseases is currently under investigation in several centers worldwide. Several cell lineages have been investigated in experimental models [21-26], and the first human case series have already been described [27-31].

Two cell sources have been used in human beings so far:

skeletal muscle (origin of myoblasts) [29] and bone marrow (source of stem cells in adults) [31,32].

The use of cells originating from the BM in experiments of neovascularization with the intracoronary, transendocardial, and transepicardial routes has been consistent in the international literature. In experimental models of acute and chronic myocardial ischemia [22,33-43], the implantation of bone marrow mononuclear cells (BMMC) has been able to improve myocardial contraction and perfusion [22,41,42,44,45]. Such results have been reproduced in a recent clinical phase-1 study in human beings [30,31,46-49], including our previous publication when the safety of that procedure was assessed [50].

This study aimed at assessing clinical evolution up to 6 months after autologous transendocardial transplantation of bone marrow mononuclear cells (ATTBMMC) based on the hypothesis that that treatment can cause neovascularization, and, consequently, result in improvement of the symptoms and exercise capacity in patients with severe ischemic heart disease and contraindication to other alternatives of revascularization [48].

Methods

This prospective nonrandomized study comprised 21 patients with severe ischemic heart disease and no other therapeutic option of percutaneous or surgical myocardial revascularization (tab. I). The detailed methodology of this study has been recently published [50].

The inclusion criteria were as follows: 1) chronic coronary artery disease with reversible perfusion defect detected on myocardial scintigraphy (SPECT); 2) left ventricular ejection fraction (LVEF) < 40%; 3) ineligibility for percutaneous or surgical myocardial revascularization assessed on coronary angiography; and 4) signed written informed consent. Ineligibility for the surgical or percutaneous myocardial revascularization procedures was determined by the following 2 committees: a surgical committee formed by 2 cardiovascular surgeons and one clinical cardiologist, and an interventional committee formed by 2 interventional cardiologists and one clinical cardiologist. The exclusion criteria were as follows: 1) difficulty in obtaining vascular access for percutaneous procedures; 2) history of neoplasia or any other previous or concomitant comorbidity that could impact on the short-term survival of the patient; 3) malignant ventricular arrhythmias; 4) LV aneurysm; 5) unexplained laboratory abnormalities; 6) bone tissue with an abnormal radiological aspect; 7) primary hematological disease; 8) acute myocardial infarction in the 3 months preceding the inclusion in the protocol; 9) presence of intraventricular thrombus; 10) hemodynamic instability during the procedure; 11) atrial fibrillation; or 12) any condition that, according to the investigator, could jeopardize the patient's health.

The symptoms of angina and heart failure were categorized according to the Canadian Cardiovascular Society Classification (CCSC) and the New York Heart Association (NYHA) classification, respectively.

The following assessments were performed: treadmill testing, using the peak VO_2 for evaluating maximum oxygen consumption; uni- and 2-dimensional Doppler echocardiography, using the Simpson technique; myocardial scintigraphy with pharmacological stress with dipyridamole; measurement of the Brain Natriuretic Peptide (BNP) level; electrocardiography; and clinical evaluation.

The patients in the treated group underwent invasive assessment by using coronary angiography, left ventriculography, and electromechanical mapping immediately before the injection procedure and 4 months after it. The NOGA® mapping system (Cordis®, Miami Lakes, Fla, USA) was used for the electromechanical mapping aiming at identifying the viable myocardial areas (unipolar voltage > 6.9 mV) and guiding the injection of the solution of the mononuclear cells.

The patients in the control group underwent only the noninvasive examinations described in this study. Both the treated and control groups underwent reevaluation in the 2-month and 6-month follow-ups, by using the same procedures of the initial evaluation. It is worth emphasizing that all patients were on full clinical treatment at the maximum tolerated doses at the time of inclusion in the protocol.

Aspiration and isolation of the bone marrow mononuclear cells – Approximately 4 hours before the transendocardial injection of mononuclear cells, a 50-mL bone marrow aspirate was obtained from the posterior iliac crest of the patients in the treated group, who were under local anesthesia in association with sedation/analgesia. The mononuclear cells were isolated by density gradient on Ficoll-Paque Plus® (Amersham Biosciences), exhaustively washed with heparinized saline solution containing 5% human albumin, and then filtered by using a 100-μm nylon sieve to remove the cell aggregates. A small fraction of the cell suspension was used for cytometry and assessment of cell viability through the exclusion method with trypan blue; cell viability was greater than 90% (96.2±4.9%), assuring the quality of the suspension.

Characterization of the markers of leukocytic differentiation by use of flow cytometry and functional assessment were also performed in a fraction of the cells obtained. The clonogenic capacity of the hematopoietic progenitors was assessed by the units forming colonies of granulocytes and macrophages, as previously described [51]. Bacterial and fungus cultures of the cell preparations clinically used were performed.

Bone marrow mononuclear cell implantation - The patients in the treated group were transferred to the cardiovascular intervention laboratory approximately 3 hours before the beginning of the procedure to undergo coronary angiography and left ventriculography followed by electromechanical mapping, aiming at identifying viable myocardial areas defined as having preserved unipolar voltage (> 6.9 mV) and decreased mechanical activity (local linear shortening < 12%) [52,53]. The transendocardial injections with the NOGA Myostar® cardiology catheter were performed at points with the above characteristics.

Before the procedure, each injection point was carefully assessed by using the following criteria: 1) perpendicular position of the catheter in the left ventricular wall; 2) excellent stability of the catheter (loop stability < 4 mm); and 3) presence of ventricular extrasystole when the needle reached the myocardium. Fifteen 0.2-mL injections were performed (25.5±6.3 x 10^6 cells/patient).

The differences in the demographic characteristics between the groups were assessed by use of the chi-square test. The Fisher exact and T tests were used for the discrete and continuous variables, respectively. The changes occurring between the initial phase and the 2-month and 6-month follow-ups in the treated group and control group were compared by use of the repeated-measures ANOVA. Assessment of the changes occurring throughout

the 6-month follow-up in the treated group as compared with those in the control group was performed by use of the repeated-measures ANOVA model, including the interaction between both groups with Kruskal-Wallis post hoc analysis for nonparametric variables and for continuous variables with asymmetric distribution. Post hoc analysis with Bonferroni adjustment was used for continuous variables with normal distribution. A P value < 0.05 was considered statistically significant.

The committee on ethics and research involving human beings of the Hospital Pró-Cardíaco and the National Committee on Ethics and Research (Comissão Nacional de Ética em Pesquisa - CONEP) approved the study protocol.

Results

The clinical characteristics did not differ between the groups (table I). The patients were treated according to the Brazilian guidelines for heart failure [54]. All patients used angiotensin-converting enzyme inhibitors (ACEI) or angiotensin-receptor inhibitors. Seventy-one percent of the patients in the treated group and 50% of the patients in the control group were on beta-blockers. No difference was observed in regard to the use of nitrates, ACEI, beta-blockers, and diuretics during the 6-month follow-up, as shown in table II and figure 1.

The characteristics of the cell population are described in table III. Cell viability was greater than 95% (96.2±4.9%), assuring the quality of the cell suspension. The fungus and bacterial cultures were negative. The total duration of the injection procedure was 81±19 minutes. The electromechanical maps had 92±16 points.

No major complications related to the procedure occurred. One patient had a transient episode of pulmonary congestion that was treated with diuretics and dobutamine. No sustained arrhythmia was observed during the procedure. No pericardial effusion was detected on the serial echocardiographies performed within the 48 hours following the procedure. All patients were discharged from the hospital 48 hours after the injection procedure as predicted in the protocol.

One patient in the control group died 2 weeks after being admitted into the study and was not included in the analysis. In the 14th week of follow-up, one patient in the treated group experienced sudden death, preceded by strong chest pain. The family did not consent to the autopsy.

Table I - Clinical characteristics of the treated and control groups

	Treated Group	Control Group	p
N	14	7	
Age	56.9 ± 9.8	64.3 ± 7.3	0.1
Sex (% male)	85.7	90	0.53
Hypertension (%)	64.3	70	0.74
Diabetes (%)	28.6	70	0.35
Hypercholesterolemia (%)	78.6	70	0.35
Smoking (%)	7.1	0	0.47
Previous AMI (%)	100	100	1
Previous PTCA (%)	7,1	40	0.09
Previous MR (%)	64.3	90	0.61
Previous stroke (%)	28.6	10	0.26
Peripheral vascular disease (%)	57.1	60	0.66
Chronic renal failure (%)	14.3	10	1
Multivessel disease (%)	100	100	1

The patients in the treated group had fewer symptoms of heart failure and angina after 2 and 6 months of follow-up as compared with those in the control group (P=0.0001 and 0.008, respectively).

No severe ventricular arrhythmia was observed on the 24-hour Holter monitoring performed immediately after the procedure and during the 2- and 6-month follow-up periods. The number of ventricular extrasystoles tended towards a reduction from 4.445± 8.512 during 24 hours to 941±1.244 during 24 hours (P=0.08), and this pattern was maintained until the sixth month of follow-up. The percentage of variation in VES was significant neither in the 2-month follow-up (from 3.8±6.8% to 0.9±1.14%), nor in the 6-month follow-up (from 3.8±6.8% to 0.95±1.28%).

During the 6-month follow-up, of all laboratory tests (table IV), only the creatinine levels varied between the control and treated groups. The creatinine levels increased significantly in the control group as compared with those in the treated group (P= 0.02). The PCRt levels in the initial assessment and in the 2-month and 6-month follow-ups did not significantly differ between the 2 groups (P=0.8). An increase in the BNP levels occurred in

Table II - Clinical treatment

	Initial % (mg/day)	6 months % (mg/day)	p
ACEI + ATI1			
Control group	85.7 (63.8±52.0)	85.7 (62.6±54.0)	1.0
Treated group	85.7 (71.2±77.3)	92.9 (70.3±83.6)	0.3
p	0.60		
Nitrate			
Control group	85.7 (67.1±45.7)	85.7 (72.9±50.0)	1.0
Treated group	92.8 (44.0±35.0)	85.7 (48.3±37.6)	0.9
p	0.90		
Beta-blocker			
Control group	42.9 (17.1±9.4)	57.1 (16.2±8.4)	0.5
Treated group	71.4 (11.5±7.3)	64.3 (16.7±9.4)	0.7
p	0.90		
Diuretics			
Control group	71.4 (85.0±53.0)	57.2 (90.0±50.0)	0.7
Treated group	85.7 (60.0±24.5)	71.4 (40.0±28.3)	0.6
p	0.70		
Ca2+ Antagonist			
Control group	14.3 (¥)	28.6 (¥)	0.5
Treated group	21.4 ( )	21.4 ( )	1.0
p	0.60		

(¥) Only 2 patients in the control group used calcium antagonists. The first patient was treated during the entire protocol with diltiazem, 180 mg/day. Amlodipine, 10 mg/day, was added to the treatment of the second patient from the fourth month onwards for blood pressure control (BP). Only 3 patients in the treated group were on calcium antagonists. Two patients were using diltiazem (180 mg/day and 60 mg/day), and one patient was using amlodipine, 10 mg/day for BP control. ACEI - angiotensin-converting enzyme inhibitors, ATI1 - angiotensin-receptor inhibitors.

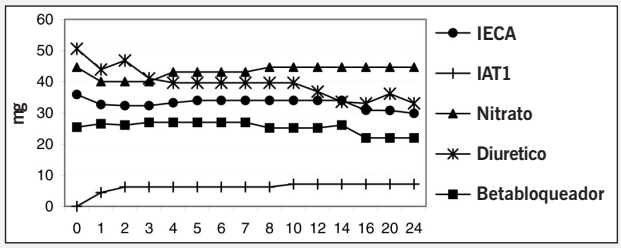

Fig. 1 - Weekly evolution of the medicamentous therapy in the treated group. ACEI - angiotensin-converting enzyme inhibitors, ATI1 - angiotensin-receptor inhibitors.

Table III - Characteristics of the cell population

Cell population and phenotype	Percentage of injected cells	Number of injected cells (x 10^3)/ mm^2
Progenitor hematopoietic cells ($CD45^{lo}CD34^+$)	2.4 ± 1.8*	57.4 ± 61.4*
Early progenitor hematopoietic cells ($CD45^{lo}CD34^+HLA\text{-}DR^-$)	0.1 ± 0.06	2.1 ± 1.8
$CD4^+$ T cells ($CD45^+CD3^+CD4^+$)	29.5 ± 10.1	537.0 ± 265.7
$CD8^+$ T cells ($CD45^+CD3^+CD8^+$)	17.9 ± 5.4	311.0 ± 221.6
B cells ($CD45^+CD19^+$)	19 ± 10	232.5 ± 174.8
Monocytes ($CD45^+CD14^+$)	8.2 ± 2.2	202.8 ± 161.0
NK cells ($CD45^+CD56^+$)	1.14 ± 0.8	21.2 ± 13.5
Functional assessment	Number of colonies / 10^6	Number of injected cells BMMC(x 10^3) / mm^2
CFU-F (colony-forming unit - fibroblast)	3.2 ± 2.8	0.2 ± 0.2
CFU-GM (colony-forming unit – granulocyte-macrophage)	792.6 ± 84.8	16.4 ± 18.5

Characteristics of the cell population and phenotype. * Results of the cell phenotype of the 14 patients undergoing the procedure of bone marrow mononuclear cell injection, except for $CD34^+CD45^{lo}HLA\text{-}DR^-$ assessed in 13 patients; $CD45^+CD14^+$ was assessed in 11 patients; and $CD45^+CD56^+$ was assessed in 9 patients.

Tabela IV - Evolution of the laboratory parameters assessed initially and 2 and 6 months after the BMMC transplantation

	Treated group	Control group	p
N	14	7	
Creatinine (Pre) (mg/dL)	1.17±0.32	1.58±0.9	ns ^{P3*}
Creatinine (Post 2m) (mg/dL)	1.1±0.3	1.78±0.9	
Creatinine (Post 6m) (mg/dL)	1.1±0.27	1.8±1.1	
p	0.23 ^{P*}	0.05 ^{P**}	0.02 ^{P4*}
PCRt (Pre) (mg/dL)	0.93±0.70	0.76±0.53	ns
PCRt (Post 2m) (mg/dL)	1.03±1	0.69±0.6	
PCRt (Post 6m) (mg/dL)	0.96±0.44	0.94±0.65	
p	0.69	0.96	0.8
BNP (Pre) (pg/mL)	328.1±410.7	466±415	ns
BNP (Post 2m) (pg/mL)	274±271	694±264	
BNP (Post 6m) (pg/mL)	443±404	657±245	
p	0.43	0.3	0.003

BNP = Brain Natriuretic Peptide; ns = nonsignificant; P^* = repeated-measures Anova over a 6-month follow-up period in the treated group; P^{**} = repeated-measures Anova over a 6-month follow-up period in the control group; P^{3*} = univariate analysis of the difference between the treated and control groups on initial assessment before the procedure of bone marrow mononuclear cell injection; P^{4*} = repeated-measures Anova over a 6-month follow-up period in the treated group as compared with that of the control group, including the interaction between both groups.

the control group as compared with those in the treated group in the 6-month follow-up (P=0.003).

In the initial assessment, ventricular geometry differed significantly between both groups. The control group showed smaller end-systolic and diastolic volumes (P<0.001) and a greater initial EF (P=0.054). However, in the 6-month follow-up, EF significantly decreased in the control group (P=0.05), which did not occur in the treated group. The cavitary volumes remained unaltered in both groups.

The peak VO_2 was similar in both groups in the initial assessment, and, after 2 months, a significant increase occurred in the treated group (from 17.96±8.78 to 23.40±8.30; P= 0.01), which remained (24.20±7.50) in the 6-month follow-up (P=0.05). This, however, was not observed in the control group (table V). This variation in the peak VO_2 was not sufficient to generate a significant statistical difference between both groups by the end of the 6-month follow-up.

The size of the total defect of reversible perfusion (TDRP) (ischemia) and the percentage of defect of rest perfusion (PDRP) with 50% activity (scar) was similar in both groups in the initial assessment. No variation in 50% PDRP was observed in the 2-month and 6-month follow-ups in both groups. In the treated group, a significant absolute reduction of 10% in the TDRP (P=0.022) was observed in the end of 2 months (73% relative reduction), which significantly differed from that in the control group. After 6 months, this absolute reduction in the ischemic area lost 4% of its initial impact, but maintained a 6% absolute reduction (in regard to the initial assessment) (table V) and a significant difference in regard to the control group (P=0.05). Figure 2 depicts an example of resolution of inferolateral ischemia in a treated patient.

Discussion

This study reports, for the first time, the medium-term evolution (6 months) of patients with severe ventricular dysfunction undergoing autologous transendocardial transplantation of bone marrow mononuclear cells by use of transendocardial injections. The results suggest that BMMC injection is a safe therapy, both in the short run [50] and in the 6-month follow-up.

Studies with experimental models have reported the possibility of neovascularization in ischemic tissues by use of BMMC transplantation. Our group has carried out a study in rats showing the capacity of bone marrow cells to generate an angiogenic process, which has been demonstrated by functional and histopathologic data [55]. Similar results have been reported by other authors, such as Fuchs and Kawamoto [22, 40], who studied pigs with chronic myocardial ischemia induced by implantation of constrictors in the coronary artery, which then underwent BMMC transplantation. Before one month, the animals underwent assessment, which included histopathological analysis, and the authors reported an improvement in myocardial perfusion related to the development of new vessels. Therefore, in our study, angiogenesis may have been one of the mechanisms to explain the improvement in myocardial perfusion observed in the injected areas in the treated group. Although not described in the results, most patients reported an improvement in symptoms from the second week onwards, which could only be demonstrated after 8 weeks according to the study's design.

The objective demonstration of an improvement in perfusion is fundamental when dealing with techniques of angiogenesis, which, according to data in the literature, are highly susceptible to the placebo effect [13-19]. Recently, improvement in the symptoms of patients undergoing transmyocardial laser revascularization have been reported [7-12]; those data, however, could not be confirmed in randomized, double-blind studies [56,57]. It is worth noting that, contrary to that which has been observed with cell therapy, no improvement has been found in myocardial perfusion scintigraphy

Table V - Evolution of the clinical and functional parameters initially, and 2 and 6 months after BMMC transplantation

	Treated group	Control group	p
N	14	7	
NYHA (Pre)	2.21±0.89	2.71±0.75	ns^{P3*}
NYHA (Post 2m)	1.14±0.36	2.57±0.53	
NYHA (Post 6m)	1.30±0.60	2.43±0.53	
p	0.001^{P*}	0.75^{P**}	0.0001^{P4*}
CCSC* (Pre)	2.64±0.84	2.57±0.97	ns
CCSC (Post 2m)		1.29±0.60	2.29±0.95
CCSC (Post 6m)		1.38±0.50	2.14±0.38
p	0.0001	0.23	0.008
Peak VO_2 (Pre)	17.96±8.78	17.50±6.80	ns
Peak VO_2 (Post 2m)	23.40±8.30	16.90±10.30	
Peak VO_2 (Post 6m)	24.20±7.50	15.30±8.00	
p	0.05	0.9	0.08
METs (Pre)	5.09±2.5	5.07±1.96	ns
METs (Post 2m)	6.68±2.35	5.16±2.45	
METs (Post 6m)	7.19±2.4	5.08±2.04	
p	0.04	0.9	0.02
TDRP** (Pre)	15.15±14.90	20±25	ns
TDRP (Post 2m)	4.50±10.60	40±38	
TDRP (Post 6m)	8.75±9.90	19.60±26	
p	0.13	0.23	0.05
PDRP*** (50%) (Pre)	40.80±11	35.80±9.50	ns
PDRP (50%) (Post 2m)	38.80±8.80	36±11.40	
PDRP (50%) (Post 6m)	38.80±7.0	36±8.0	
p	0.44	0.4	0.19
EF (%) (Pre)	30±5.56	36±11.70	0.054
EF (%) (Post 2m)	35.60±7.90	29.40±7.60	
EF (%) (Post 6m)	32.83±6.97	28.60±4.0	
p	0.18	0.05	0.03
ESV (Pre)<0.001	146.80±53	96.70±19.60	<0.001
ESV (Post 2m)	123±47.90	103.90±25.60	
ESV (Post 6m)	133.25±46.20	103.90±28.90	
p	0.91	0.43	0.003
EDV (Pre)	211±77	141.80±22.40	<0.001
EDV (Post 2m)	189±67.50	146.30±29.10	
EDV (Post 6m)	209±56	146.60±43	
p	0.89	0.50	0.0001

CCSC - Canadian Cardiovascular Society Classification; TDRP - Total defect of reversible myocardial perfusion; PDRP - Percentage of defect of myocardial rest perfusion; ns - nonsignificant; P* = repeated-measures Anova over a 6-month follow-up period in the treated group; P** = repeated-measures Anova over a 6-month follow-up period in the control group; P^{3*} = univariate analysis of the difference between the treated and control groups on the initial assessment before the procedure of bone marrow mononuclear cell injection; P^{4*} = repeated-measures Anova over a 6-month follow-up period in the treated group as compared with the control group, including the interaction between both groups.

after transmyocardial laser revascularization. Differently, our group and others have reported an improvement in the myocardial perfusion pattern in patients undergoing bone marrow mononuclear cell transplantation [48-50].

Preliminary clinical results in patients with recent acute myocardial infarction have suggested that a reduction in the area of fibrosis occurs after therapy with bone marrow mononuclear cells [30,31]. However, no clinical data exist suggesting the occurrence of that type of effect in the model of patients with severe ventricular dysfunction reported in our study. On the contrary, in addition to

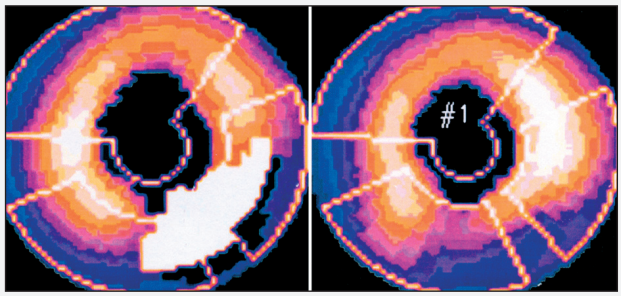

Fig. 2 - Example of myocardial scintigraphy after treatment with BMMC. The white area represents the area of initial ischemia that disappears in the 2-month follow-up.

the fact that those patients did not have an improvement in their defect on rest scintigraphy, which might suggest a myogenic effect, it is worth noting that an improvement occurred in ESV, but not in EDV, suggesting a better contractile performance of the viable areas rather than the appearance of new viable areas. Because the cells were injected only in areas of ischemic myocardium, it is reasonable to imagine that that microenvironment provided a strong angiogenic signal to transplanted cells, such as through the presence of the hypoxia inducible factor (HIF-1), generating the vascular endothelial growth factor (VEGF). If this hypothesis is confirmed, accuracy on the technique of cell delivery may be important to reach the therapeutic objectives of each patient.

Although the small number of patients and the open design of the study may justify the statistical differences observed when comparing some variables (Hawthorne effect) [58], data presented have suggested that the results found may have been secondary to an improvement in myocardial perfusion.

In this initial prospective, nonrandomized study in patients with coronary artery disease and severe ventricular dysfunction and no other treatment option, severe adverse effects related to the procedure were observed neither at the time of hospital admission nor in the 6-month follow-up. During the follow-up period, neither deterioration in the mechanical and perfusion parameters in the injected areas, nor any sign of dysfunction in the conduction system was observed. Improvement in symptoms, in cardiac function, and in myocardial perfusion was observed in the treated group as compared with that in the control group. We believe that a huge clinical potential exists for this technique. Further investigation conducted in larger randomized studies is required.

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