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AUTOLOGOUS STEM CELL THERAPY FOR ACUTE MYOCARDIAL INFARCTION WITH SEVERE SYSTOLIC DYSFUNCTION - PROTOCOL AND FEASIBILITY AT 1 MONTH FOLLOW-UP - BUCHAREST EXPERIENCE

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ABSTRACT. Our aim was to assess the feasibility and safety within first month after autologous bone marrow stem cell therapy in patients with acute myocardial infarction (AMI) and severe systolic dysfunction. Twelve patients were included. All the patients were treated in accordance with existing guidelines. In the stem cell treated group, collection of 50 ml of bone marrow aspirate was performed; after density gradient separation, the mononuclear bone marrow cell suspension was delivered via intracoronary route.

At 1 month follow-up, there were no significant differences between groups regarding systemic inflammatory response, malignant arrhythmias or deaths. Moreover, in stem cell treated group we observed an antiremodelling effect along with improvement of left ventricle systolic function.

The autologous bone marrow stem cell therapy has proven to be a feasible and safe method of treatment, but larger studies are required in order to reveal the intimate mechanisms of action.

Keywords: autologous bone marrow stem cells, acute myocardial infarction, left ventricular systolic function, MACE, left ventricular volumes.

INTRODUCTION

In spite of modern treatment, acute myocardial infarction is still an important cause of mortality and morbidity worldwide. The brutal injury of myocardium leads to a sudden augmentation of loading conditions and initiates a process of unfavorable ventricular remodelling, which affects not only the necrotic muscle, but the border zone and the distant myocardium as well (McKay *et al.,* 1986). It starts immediately after the acute event and evolves for weeks or even months. This adverse remodelling process, along with the impossibility to maintain normal wall stresses lead to gradual dilatation, recruitment of border zone myocardium into the scar and decline of contractile function. As a result, heart failure occurs, with all its consequences (Pfeffer *et al.*, 1990). Prompt reperfusion of the culprit coronary arteries is a key factor in rescuing ischemic heart muscle and limiting the magnitude of necrosis. Still, a significant proportion of patients suffering an acute myocardial infarction (AMI) undergo this remodelling process. Finding new therapeutic strategies to limit this adverse remodelling process and thus to improve the outcome of patients suffering a major acute myocardial infarction is a perpetual quest.

The aim of different types of treatments (pharmacological, interventional or surgical) is to prevent, slow or reverse cardiac remodelling. But, due to the low intrinsic regenerative potential of the heart and the fact they do not address the fundamental issue of cell loss, these therapies have only a limited capability to improve cardiac function. The discovery made by Asahara and colleagues (1999) that postnatal vasculogenesis exists, provided new insights into mechanisms of cardiac repair. These data, as well as the original work of Orlic and collaborators (2001) that showed improved cardiac function in a mouse model of myocardial ischaemia, in which grafted cells were seen in the infarcted region and differentiated into cardiomyocytes, are the basis of bone marrow stem cells use in ischemic cardiac disease.

Stem cell (SC) therapy intend to regenerate structurally and functionally the injured heart by preventing and improving the cardiac remodelling; this innovative therapy is used additional to current guidelines recommendations.

OBJECTIVES

Our aim was to assess the feasibility and safety within first month after autologous bone marrow stem cell therapy in patients with AMI and severe systolic dysfunction.

MATHERIALS AND METHODS

Patient selection

This prospective pilot - study was conducted on a total of 12 patients with acute myocardial infarction with ST-segment elevation (STEMI) and angioplasty with stent implantation, admitted to the Cardiology Department of Clinical Emergency Hospital of Bucharest. These patients were followed - after a strict protocol- with specific biological and imaging parameters in order to establish a correlation between autologous bone marrow stem cell (ABMSC) therapy and the subsequent development of patients.

Inclusion criteria were: age between 18 and 81, STEMI with symptoms onset within the prior 12 hours, myocardial revascularization by angioplasty, impaired left ventricular ejection fraction (LVEF $\leq$ 40%), and signed informed consent to participate in the study.

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The main exclusion criteria were: pregnancy or lactation; hemodynamic instability, NYHA class IV; severe valvular disease; intra-ventricular thrombosis; primary haematological disease; autoimmune disease; malignant tumors; renal, hepatic or respiratory severe impairment; active infection (viral or bacterial); fever> 38 °C for two consecutive days; alcohol or drug dependence; any other severe co-morbidity meant to alter patient prognosis; refuse to participate in the study; presentation to hospital beyond the therapeutic window.

Study design

Patients were assigned to either the experimental group (undergoing autologous bone marrow stem cell transplantation, 5 patients) or control group (those who refused cell transplantation, 7 patients).

Complete clinical examinations and biochemical analysis of blood samples were performed at baseline and 1 month after STEMI, as well as 2D echocardiography. In addition, at 1 month follow-up, 24h ECG monitoring was carried out in order to objective malignant arrhythmias.

Patients in both groups received standard therapy according to up to date guidelines and were evaluated using the same methods. Neither bone marrow aspiration nor sham injection was performed in the control group.

Cell preparation and administration

After 6-13 days from myocardial infarction a total of 50 ml of bone marrow was aspirated into heparin-treated syringes from the iliac crest with the use of local anaesthesia. The bone marrow aspirate was stored at room temperature together with 10 ml of venous blood used to produce patients' own serum. Progenitor cells were isolated and enriched with the use of Ficoll centrifugation procedures. The procedure was made in sterile conditions in Cell Culture Laboratory within Clinical Emergency Hospital of Bucharest. The Laboratory is equipped according to international standards to fulfil all conditions for a "clean room". Strategy applied to determine the absolute number of cells $CD34^{+}CD45^{dim}$ (true stem cells) in the blood marrow - before or after separation of mononuclear cells - is an adaptation protocol for assessment of cells $CD34^{+}$ in units of thawed cord blood (Lanza & Saccardi, 2011). After the catheterization of culprit artery, blood flow was blocked by inflating the balloon for 2-3 minutes and cell suspension (12 ml divided in 3 doses) was injected distal to the balloon to allow adherence and trans-endothelial migration of cells.

Statistical analysis

Data are expressed as percentages for categorical variables and as mean ± standard deviation for numeric variables. Continuous variables were tested for normal distribution by Kolmogorov-Smirnov test using Lilliefors' correlation. To compare groups we used Student test for quantitative variables with normal distribution, respectively chi-square test for nominal or ordinal variables. All tests were two-sided. Statistical analysis was performed using SPSS software version 15.0 or Microsoft Excel for Windows 7.0. For all tests, $p < 0.05$ was considered statistically significant.

RESULTS

As depicted below, there were no significant differences between the two groups of patients regarding the baseline characteristics (socio-demographic features, personal history, biological parameters, myocardial dysfunction, revascularization strategy and angiographic features of coronary circulation, echographic parameters, drug therapy) (Table 1 and 2).

Table 1

Baseline characteristics of the patients and drug therapy at discharge

Characteristic	ABMSC group (N = 5)	Control group (N = 7)	p
Age-years (mean ± SD)	59.8 ± 10.28	57.8 ± 11.83	NS
BMI -kg/m^2 (mean ± SD)	27.94 ± 4.72	24.67 ± 2.57	NS
Sex			
• M	5 (100%)	5 (71.4%)	NS
• F	0 (0%)	2 (28.6%)	NS
Smoking status			
• Non-smoker	3 (60%)	2 (28.6%)	NS
• Smoker	2 (40%)	5 (71.4%)	NS
Diabetes mellitus			
• Absent	5 (100%)	6 (85.7%)	NS
• Present	0 (0%)	1 (14.3%)	NS
Dyslipidemia			
• Absent	2 (40%)	3 (42.9%)	NS
• Present	3 (60%)	4 (57.2%)	NS
Hypertension			
• Absent	2 (40%)	2 (28.6%)	NS
• Present	3 (60%)	5 (71.4%)	NS
Killip Class			
• Killip I	3 (60)	3 (42.9)	NS
• Killip II	1 (20)	3 (42.9)	NS
• Killip III	1 (20)	1 (14.3)	NS
Topol Class			
• Topol 1	2 (40)	3 (42.9)	NS
• Topol 2	1 (20)	2 (28.6)	NS
• Topol 3	2 (40)	2 (28.6)	NS
Time from pain onset to PCI (hours)	11 ± 1.73	8 ± 3.51	NS
Number of diseased coronary arteries n (%)			
• 1	2 (40)	1 (14.3)	NS

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• 2	2 (40)	4 (57.1)	NS
• 3	1 (20)	2 (28.6)	NS
Preprocedural TIMI Grade n (%)			
• TIMI 1	5 (100)	3 (42.9)	0.05
• TIMI 2	0	3 (42.9)	0.05
• TIMI 3	0	1 (14.3)	0.05
Postprocedural TIMI Grade n (%)			
• TIMI 1	1 (20)	0	NS
• TIMI 2	0	3 (42.9)	NS
• TIMI 3	4 (80)	4 (57.1)	NS
BLUSH Score n (%)	N=4	N=4	NS
• BLUSH 0	0	1 (25)	NS
• BLUSH 1	1 (25)	0	NS
• BLUSH 2	1 (25)	1 (25)	NS
• BLUSH 3	2 (50)	2 (50)	NS
Stent type n (%)			
• BMS	5 (100)	6 (85.7)	NS
• DES	0	1	NS
Drug therapy at discharge			
Aspirin	4 (80)	7 (100)	NS
Clopidogrel	5 (100)	7 (100)	NS
Oral anticoagulant	3 (60)	4 (57.1)	NS
βeta -blocker	5 (100)	7 (100)	NS
ACE inhibitor	4 (80)	6 (85.7)	NS
Statin	5 (100)	7 (100)	NS
Aldosterone antagonist	3 (60)	5 (71.4)	NS
Diuretics	4 (80)	7 (100)	NS
Digitalis	1 (20)	0	NS

SD= standard deviation; NS = insignificant (p > 0.05)

Table 2
Ultrasound parameters of left ventricular (LV) function at admission

Variable	ABMSC group (N = 5) mean± SD	Control group (N = 7) mean± SD	p
LV end-diastolic volume (ml)	147.6 ± 38.93	135 ± 40.3	NS
LV end-systolic volume (ml)	105 ± 24.5	96 ± 30.4	NS
LVEF (%)	28.4 ± 3.2	30 ± 5.7	NS

SD= standard deviation; NS = insignificant (p > 0.05)

The time interval between STEMI onset and SC injection in the culprit coronary artery was, on average, 9.2 ± 2.38 days, with a minimum value of 7 days and a maximum value of 13 days. The mean time passed between harvesting and injecting the cells was, on average, 9.8 ± 7.94 hours, varying between 6 hours (minimal value) and 24 hours (maximal value). The total number of transplanted mononuclear cells ranged between 4.25 x 10^7 (minimal value) and 5.3 x 10^8 (maximal value), with a mean of 2.49 ± 2.03 x 10^8 cells. As for the $CD34^+$ cells percentage, it varied between 0.23% (minimal value) and 1.2% (maximal value), with an average value of 0.71 ± 0.39%; the total number of $CD34^+$ cells was, on average, 2.02 ± 1.67 x 10^6 cells, with a minimal value of 9.3 x 10^4 cells and a maximal value of 3.65 x 10^6 cells.

Table 3 summarizes the evolution of the inflammatory parameters at 1 month follow-up, with no statistically difference between groups except erythrocyte sedimentation rate (ESR) level at 1 month, when in ABMSC group the ESR was significantly lower than the values of control group (p = 0.01).

Table 3
Evolution of the inflammatory syndrome within 1st month after STEMI

Variable	Baseline		One month follow-up	
	ABMSC group (N = 5) mean± SD	Control group (N = 7) mean± SD	ABMSC group (N = 5) mean± SD	Control group (N = 7) mean± SD
WBC (/mm^3)	11100 ± 1254.99	16828 ± 8108	7980 ± 890	8612 ± 1624
Fibrinogen	717 ± 311.1	752 ± 169.47	472.6 ± 149.24	548.5 ± 58
ESR (mm/h)	35.5 ±13.69	50 ± 17.32	16 ± 8.16	33 ± 9.85

As illustrated in table 4, at 1 month follow-up, there were no significant differences between groups regarding the absolute changes in LV volumes. Still, the absolute increase in LVEF was significantly greater in the ABMSC group than in the control group (p < 0.05).

Table 4
Absolute changes in LV volumes and LVEF at 1 month after STEMI

Variable	ABMSC group (N = 5) mean± SD	Control group (N = 7) mean± SD	p
LV end-diastolic volume (ml)	4.8 ± 20.45	11.71 ± 19.49	NS
LV end-systolic volume (ml)	-4.30 ± 14.6	7.79 ± 19.12	NS
LVEF (%)	5.00 ± 1.83	-0.50 ± 4.26	0.04

NS = insignificant (p > 0.05)

Regarding the safety of ABMSC therapy, the occurrence of malignant arrhythmias, re-hospitalization for heart failure and death, did not differ significantly between the two groups during follow-up.

Table 5
Incidence of MACE

Variables	ABMSC group (N = 5) mean± SD	Control group (N = 7) mean± SD	p
Malignant arrhythmias n (%)	0	2 (28.6)	NS
Hospitalization for heart failure n (%)	0	0	NS
Death n (%)	0	0	NS

NS = insignificant (p > 0.05)

DISCUSSION

Studies conducted so far showed heterogeneous results regarding the beneficial effect of ABMSC therapy in patients with AMI. Most of these studies have focused on end-point assessment starting 3 to 6 months since acute coronary event occurred. From 33 randomized clinical trials involving a total of 1765 patients (Clifford *et al.*, 2012), only 2 studies evaluated the outcome of cell therapy starting with the first month after transplantation (Cao *et al.*, 2009; Wöhrle *et al.*, 2010).

The main objective of our study was to assess the feasibility and safety of cell therapy within 1st month after STEMI in patients with moderate-to severe left ventricle systolic dysfunction, as regards to inflammatory syndrome and impact on major adverse cardiac events such as: malignant ventricular arrhythmias, hospitalization for heart failure and death. We were also interested in seeing if there is an early beneficial effect of ABMSC transplant concerning the left ventricle remodelling and systolic function.

The study included 12 patients with STEMI and LVEF < 40%, mean age 58.67 ± 10.76 years, mostly men (83.3%) divided in two groups: the experimental group (5 patients) and the control group (7 patients) (Table 1). These characteristics are in accordance with literature data, which reported a mean age between 48 (Zhukova *et al.*, 2009) and 67 years (Piepoli *et al.*, 2010), most patients being males within a range between 56.3% (You *et al.*, 2008) and over 90%, respectively (Meluzin *et al.*, 2008; Quyyumi *et al.*, 2011).

To identify a possible persistence/worsening of inflammatory syndrome due to cell therapy, we observed the dynamics of the erythrocyte sedimentation rate, leukocyte and fibrinogen. The application of significance tests for independent samples revealed no significant difference between the two groups regarding inflammatory syndrome at 1 month post-infarction, the only exception being the ESR level, when in the experimental group the ESR was significantly lower than the values of control group ($p = 0.01$). Therefore, cell therapy has been shown not to maintain/exacerbate post-infarction inflammatory syndrome (Table 3).

The effect of cell therapy on cardiac remodelling and LV systolic function have been widely studied starting with few months after STEMI, but there are little data concerning the results within 1st month. The only available data in relation to this issue have been provided by 2 studies (Cao *et al.*, 2009; Wöhrle *et al.*, 2010).

Cao and colleagues assessed the results of bone marrow mononuclear cells transplantation starting with the 1st month after infarct. They reported a decrease in end-systolic volume, as well as an improvement in LVEF within 1st month in both the ABMSC and the control group, but the difference between groups becoming statistically significant at 6 months. There was no significant difference among groups in terms of LV end-diastolic volume change regardless the follow-up visit (neither 1 month nor 6 months).

Woehrle and collaborators reported no difference between groups as regards to LV volumes and LVEF during the follow-up period (1 to 6 months). Unlike the above mentioned study, which included patients with impaired systolic function (mean LVEF < 40% in both groups), this one included patients with preserved LVEF (mean LVEF in control group 55.7±9.4% vs 53.5±9.3% in SC group). This disparity in baseline systolic function (impaired LVEF vs. preserved LVEF), as well as diverse assessment methods (2D echography vs. cardiac magnetic resonance imaging) could explain the dissimilar results.

In our case, even though the absolute change regarding the LV volumes between the two groups of patients at 1 month follow-up fell beneath the limit of statistical significance, we noticed a lower increase of LV end-diastolic volume (4.8 ± 20.45 ml vs. 11.71 ± 19.49 ml) and a mild decrease of left ventricular end-systolic volume (-4.30 ± 14.6 ml vs. 7.79 ± 19.12 ml) in ABMSC group compared with the control group. This effect on LV volumes was also reflected on the LVEF: LVEF absolute change assessed by 2D echography registered, at 1 month follow-up, an ascending evolution in the stem cells treated group, while in the control group the same variable decreased, the difference being statistically significant (5.00 ± 1.83% vs. -0.50 ± 4.26%, $p = 0.04$).

Another parameter followed in regard to the cell transplantation in STEMI patients was the arrhythmic potential. Most candidates for cell therapy are patients at high risk of developing severe rhythm disturbances (patients with AMI and depressed systolic function). In our study, at 1 month follow-up, the 24 hours Holter ECG recording showed the presence of significant arrhythmias in 2 control patients, while no patient in the experimental group experienced severe arrhythmias at the aforementioned control visit (Table 5); there were no statistically significant differences in arrhythmias in patients receiving ABMSC compared with control patients. Our results are consistent with those reported in the meta-analysis by Clifford *et al* (2012), in which the statistical analysis revealed the lack of significant differences between the two groups as far as arrhythmic potential is concerned.

Considering the occurrence of MACE, in the case of our study, there were no significant differences between groups (Table 5) probably due to the small number of patients included and the short follow-up period; although, the literature data showed a statistically significant decrease in readmission for heart failure and a tendency toward a positive evolution regarding the mortality in the case of ABMSC patients (Delewi *et al.*, 2012).

LIMITATIONS

The main limitation of the present research is the small number of patients enrolled, thus lacking the necessary power to detect statistically significant

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differences in terms of major adverse effects (cardiovascular death, recurrent infarction, need for revascularization). Still, this pilot trial could offer necessary groundwork for future larger studies.

CONCLUSIONS

Our results indicated an early effect of autologous bone marrow stem cell therapy regarding the cardiac remodelling and systolic function in acute myocardial infarction patients and impaired left ventricular ejection fraction. Our study included a small number of patients, but demonstrates the feasibility and safety of this innovative therapeutic approach.

Nevertheless, in order to use stem cells as a current therapeutic method in clinical practice, a number of problems should be solved first. Standard criteria should be established for the implementation of therapy as well as for determining which patients should be approached and what type of stem cells would be most effective for the pathology addressed.

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