Bone Marrow Cell Transplantation in Chagas' Disease Heart Failure: Report of The First Human Experience

Fábio Vilas-Boas[1], Gilson Soares Feitosa[1], Milena B. P. Soares[2], Joel Alves Pinho-Filho[1], Augusto C. A. Mota[1], Augusto José Gonçalves Almeida[1], Marcus Vinícius Andrade[1], Heitor Ghissoni Carvalho[1], Adriano Dourado Oliveira[1], Ricardo Ribeiro-dos-Santos[2]

Hospital Santa Izabel da Santa Casa de Misericórdia da Bahia[1], Centro de Pesquisas Gonçalo Moniz - FIOCRUZ/BA[2], Salvador, BA - Brazil

Abstract

Background: Heart failure due to Chagas' disease (HFCD) is a progressive inflammatory cardiomyopathy that affects millions of individuals in Latin America. Studies using mice models of HFCD indicate that bone marrow mononuclear cell transplantation (BMCT) may reduce inflammation, fibrosis, and improve myocardial function.

Objective: The purpose of this study was to evaluate, for the first time in humans, the safety and efficacy of BMCT to the myocardium of patients with HFCD.

Methods: A total of 28 HFCD patients (mean age 52.2 ± 9.9 years) with NYHA class III and IV were submitted to BMCT through intracoronary injection. Effects on the left ventricle ejection fraction (LVEF), functional capacity, quality-of-life, arrhythmias, biochemical, immunological, and neuro-humoral parameters, were evaluated.

Results: There were no complications directly related to the procedure. LVEF was 20.1 ± 6.8% and 28.3 ± 7.9%, p < 0.03 at baseline and 180 days after the procedure, respectively. In the same period, significant improvements were observed in the NYHA class (3.1 ± 0.3 to 1.8 ± 0.5; p < 0.001), quality-of-life (50.9 ± 11.7 to 25.1 ± 15.9; p < 0.001), and in the six-minute walking test (355 ± 136 m to 437 ± 94 m; p < 0,01). There were no changes in markers of immune or neurohormonal activation. No complications were registered.

Conclusion: Our data suggest that the intracoronary injection of BMCT is safe and potentially effective in patients with HFCD. The extent of the benefit, however, appears to be small and needs to be confirmed in a larger randomized, double blind, placebo controlled clinical trial. (Arq Bras Cardiol 2011;96(4):325-331)

Keywords: Stem cells; heart failure; Chagas' disease; tissue therapy; cardiomyopathy, dilated.

Introduction

Chagas' disease is caused by a protozoan called *Trypanosoma cruzi*, and still affects millions of individuals in Latin America[1,2]. Due to the intense immigration from endemic areas, transfusion-related infection has recently been perceived as a potential threat in the USA[3]. Among its many manifestations, the cardiac involvement is very prominent, being both frequent and often disabling[4]. It has been estimated that one-fourth of the infected patients will eventually develop the advanced form of cardiac disease, which will present features of severe dilated cardiomyopathy, with frequent life-threatening arrhythmias and systemic and pulmonary embolic phenomena[5]. Macroscopic features of this condition include dilated chambers, involving all four chambers and localized areas of aneurisms, especially in the apical region. Microscopic features of this condition include widespread foci of inflammation and fibrosis[6]. The actual finding of the parasite at this late stage of the disease is infrequent, although the use of highly sensitive techniques allows the recognition of parts of the parasite in the myocardium[7]. This finding and others suggest that an autoimmune process plays a major role in the chronic phase of the disease[8,9]. In the advanced stage of the disease, the prognosis is poor and a relentless progression to the refractory condition is frequently observed, despite the current therapeutic armamentarium[10,11].

With the recent progress in bone marrow cell transplantation (BMCT) directed at ischemic heart disease[12-14], our attention was directed towards the possibility of employing the same technique in Chagas' heart disease. Experimentally, it has been demonstrated that in mice chronically infected with the Colombian strain of *T. cruzi*, the injection of autologous bone marrow mononuclear cells caused a significant reduction in inflammatory cells and interstitial fibrosis in the myocardium[15]. In a landmark paper, the simultaneous autologous transplantation of co-cultured stem cells and skeletal myoblasts in an experimental model of dilated cardiomyopathy caused by Chagas' disease has been shown to be functionally effective[16].

Mailing address: Fábio Vilas-Boas•
Av. Sete de Setembro, 4161 - Barra - 41140-900 - Salvador, BA - Brazil
E-mail: fabiovboas@cardiol.br
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Granulocyte colony-stimulating factor (G-CSF) induces mobilization of bone marrow stem cells to the peripheral blood, making a greater number of stem cells available for injury repair[17]. Thus, both the previous experience with BMCT in ischemic heart disease, and the experimental results with Chagas' disease model, set the stage for our phase 1, pilot trial, of BMCT in human cases of advanced Chagas' heart disease[18,19].

Methods

From June 2003 to June 2006, we conducted a phase-1, open-label clinical trial to test the feasibility, safety, and potential efficacy of bone marrow cell transplantation to the myocardium of patients with heart failure due to chagasic cardiomyopathy. Subjects included in the study were of both genders, ranging from 20 to 70 years, with CHF due to Chagas' disease, LV ejection fraction < 40% at the echocardiogram, functional class III and IV (NYHA), receiving optimal medical therapy for CHF, and who had remained stable in this condition for at least one month prior to the investigational procedures.

Patients were excluded if they had associated systemic conditions that could have an impact either on the treatment results or the procedure analyses. These included infections or neoplasias, autoimmune diseases, previous hematologic diseases; liver diseases; moderate renal failure (creatinine above 2 mg/dl); implantation of biventricular resynchronization pacemaker during the previous 90 days; women of childbearing potential, and patients with coronary artery disease detected by coronary angiography.

At baseline, a clinical evaluation was performed, and the following clinical data and variables were recorded: a) NYHA functional class, b) quality-of-life score based on the "Minnesota Living With Heart Failure Questionnaire"[20]; c) hematological and biochemical variables; d) twelve-lead electrocardiogram; e) transthoracic echocardiogram; f) six-minute walking test[21]; g) 24-hour Holter monitoring. After the procedure, patients were referred to the Intensive Care Unit, where they were monitored for at least 24 hours. In case of an uneventful evolution, they were transferred to the regular ward where they remained for a minimum of five days. All evaluations were repeated after 180 days.

To check for any possible myocardial damage caused by the bone marrow cell injection, serial measurements (every six hours during the first 24 hours) of myocardial damage markers (CK-MB and troponin I), as well as electrocardiograms, were performed. To investigate the development of cardiac arrhythmias as a complication of the cell injection, patients underwent ambulatory electrocardiography (Holter) 24 hours before the procedure and periodically thereafter.

The protocol was approved by the Institution's Ethics Review Board and by the Brazilian National Committee for Ethics in Research. Patients were included in the study only after being fully informed about all study procedures and signing the informed consent.

Bone marrow mononuclear cell transplantation

Patients were referred to the cath lab after overnight fasting. A total of 50 ml of bone marrow content was aspirated from every patient through five punctures in both the right and left posterior iliac crest, under local anesthesia and intravenous sedation. The harvested aspirate was filtered to remove bone marrow debris, such as fat and bone fragments (stainless steel mesh, Washington University), and centrifuged by Ficoll Hystopaque gradient (Amersham Pharmacia, a product licensed for clinical use in humans). The isolated mononuclear cell fraction was then diluted in sterile saline and centrifuged again. One sample was used for cell count and viability test.

At the end of the process, samples were diluted in 20 ml of saline. Immediately before the intracoronary injection, patients underwent left heart catheterization by the femoral approach followed by coronary angiography. Patients were excluded if a stenosis greater than 50% was seen in any coronary artery. The solution containing the cells was slowly injected, over 10 minutes, into the right and left coronary system: 10 ml into the left anterior descending artery, 5 ml into the right coronary artery, and 5 ml into the left circumflex artery.

Twenty-five days later, the patients received daily subcutaneous injections of human G-CSF (Granulokine™) for five days, at a dose of 5 μg/kg/day.

The six-minute walking test was performed according to the protocol used by Bitner et al[21] in the SOLVD study[13]. After a fifteen-minute rest, a new test was carried out, and the average of the distances walked in the two tests was used as the result. Quality of life was assessed by the "Living with Heart Failure Questionnaire". All interviews were done by the same research nurse. Echocardiographic studies were conducted by the same observer, blinded to previous exams and to the status of the patient in the protocol. Left ventricular ejection fraction was calculated according to the modified Simpson's rule[22].

TNF–alpha, IL-1-beta, IL-6, MMP-2 and MMP-9 were analyzed using commercially available immunoassay kits (Quantikine, R&D Systems, Minneapolis, MN, USA). Vasopressin was analyzed using an EIA assay kit (Ann Arbor, MI, USA). For BNP, we used a rapid fluorescence immunoassay (Biosite Diagnostics Incorporated, San Diego, California, USA). All other biochemical and hematological tests were carried out using commercially available kits.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for Windows version 9.0. Continuous variables were presented as mean ± standard deviation. Variable distribution was assessed by the Kolmogorov-Smirnov test. Since variables were not normally distributed, non-parametric tests were carried out. All comparisons were two-tailed. P values < 0.05 were considered statistically significant.

The authors had full access to the data and take responsibility for their integrity. All authors have read and agreed to the manuscript as written.

Results

During the 6-month study period, a total of 30 patients were selected. Two patients were excluded after bone marrow collection: one patient due to coronary artery disease detected by catheterization and the other due to technical

problems with the heart catheterization equipment that prevented the injection.

The baseline characteristics of the studied population are described in Table 1. As shown, patients were in the advanced stage of the disease and presented various markers of disease severity. All patients were in functional class III and IV, despite optimal treatment with a multi-drug regimen and high doses of diuretics. Digoxin was used in 95% of patients, furosemide alone or in combination was used in 85% of patients, ACEI or ARBs were used in 85%, and beta-blockers in 55%. Functional capacity was found to be low, based on the short distance covered during the six-minute walking test, and quality-of-life was severely impaired, as indicated by high scores in the Minnesota questionnaire. Ventricular function was severely depressed, with very low ejection fraction and large left ventricular diastolic diameter on the echocardiogram. The presence of hyponatremia and renal dysfunction further characterized the severe condition of this population.

There were no complications directly related to either bone marrow aspiration or cell injection. An average of 2.4 ± 1.2 x 10^8 cells was injected into the coronary arteries. Viability tests showed that 96 ± 6.5 % of the cells were viable at the time of the injection. Neither significant changes in myocardial necrosis markers in 24 hours nor electrocardiographic changes suggestive of ischemia or infarction occurred.

In order to check if the myocardial implantation of stem cells was associated with arrhythmias, the arrhythmogenic profile was evaluated through the total number of premature ventricular contractions in 24 hours, as well as their clustering and recurrence pattern. No significant change was found in the arrhythmogenic profile (Table 2).

There were four deaths during the six-month follow-up (patient 04, male, age 37, pulmonary hemorrhage and respiratory failure; patient 16, female, age 34, sudden death; patient 27, age 69, male, end-stage heart failure; patient 7, age 67, respiratory infection, complicated by renal failure and shock). No direct causal association was found between the deaths and cell transplantation. There were no neoplasias, hematological diseases, coagulopathies or any other kind of disease that could be attributed to the cell injections or their implantation.

Table 1 - Clinical and laboratory characteristics of the patients

	(n = 28)
Age (years)	52.2 ± 9.9
Male (n)	24
NYHA class (n)	
III	24
IV	4
6 min walking test (m)	355 ± 136
LV ejection fraction (%)	20.1 ± 6.8%
LVEDD (mm)	72.6 ± 8.9
QOL score	50.9 ± 11.7
Serum sodium (mEq/l)	131 ± 7.6
BUN (mg/dl)	32.1 ± 17.2
Creatinine (mg/dl)	1.4 ± 0.4

BUN - blood urea nitrogen; n - number of patients; FC - functional class (NYHA); QOL - Minnesota quality of life score; LVEDV - LV end-diastolic dimension; Values expressed in mean ± standard deviation.

Table 2 - Ventricular arrhytmias at baseline and after 6 months (number/24h)

Arrhythmia	Baseline	Six months	p value
Total PVCs	5,734 ± 6,568	6,160 ± 4,015	0.55
Non-sustained VT	61 ± 126	31 ± 42	0.70

PVCs - premature ventricular contractions; VT - ventricular tachycardia. Values expressed in mean ± standard deviation.

Ventricular function, evaluated by left ventricular ejection fraction showed a significant, although small, improvement six months after the procedure (Figure 1).

Quality of life assessed by the Minnesota Living with Heart Failure questionnaire revealed a marked improvement in the global score at 6 months (Figure 2).

Functional capacity, as measured by the distance walked during six-minute test, showed a small, but significant improvement, at six months (Figure 3).

A significant decrease in mean NYHA functional class was observed after six-months (Figure 4).

The biochemical analysis showed a marked increase in serum sodium concentration during the study follow-up (Figure 5). However, an analysis of vasopressin concentrations did not show any significant changes in its concentrations from baseline to 180 days (193 ± 53 to 182 ± 44 pg/ml, p = 0.38).

There was a trend towards an increase in BNP levels (507 ± 523 to 720 ± 515 pg/ml; p = 0.055), that was not accompanied by any other evidence of congestion or deterioration of ventricular function.

An analysis of the turnover of the extracellular matrix revealed a significant increase in MMP-2 levels (Figure 6), but no changes in MMP-9 (1.6 ± 1.8 to 2.3 ± 2.8 pg/ml, p = 0.20).

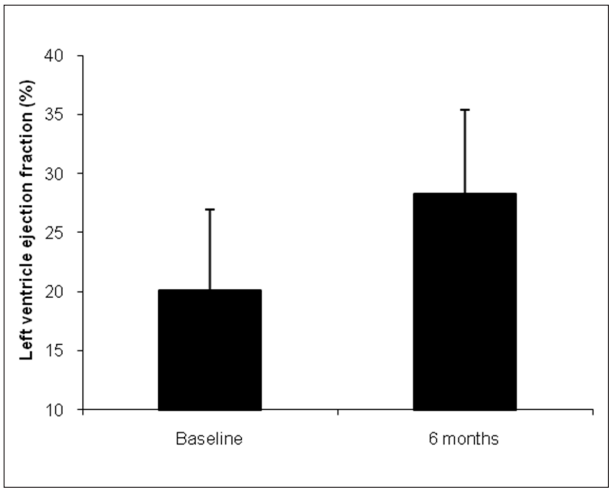

Figure 1 - *Left ventricular ejection fraction by echocardiography, before and after 6 months of BMC treatment. (Wilcoxon, P = 0.023).*

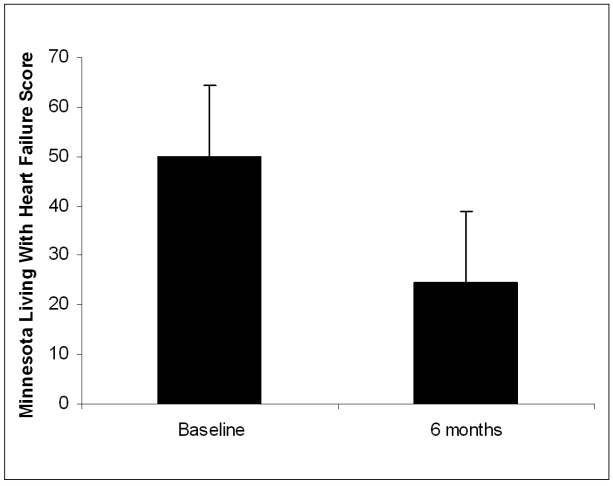

Figure 2 - Quality of life score (Minnesota questionnaire). (Wilcoxon, P < 0.001).

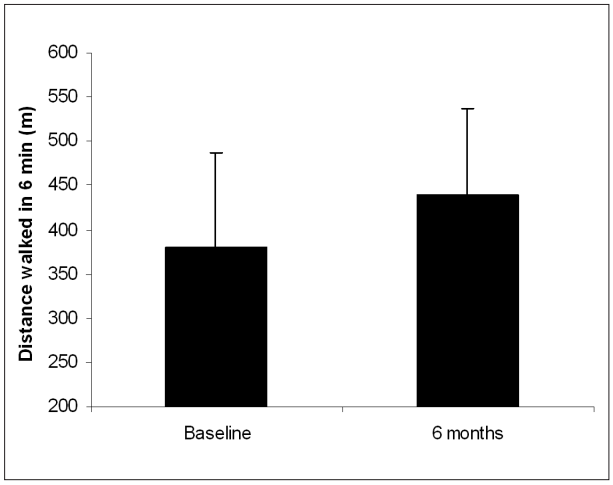

Figure 3 - Distance covered in the six-minute corridor walking test, before and after BMC treatment. (Wilcoxon, P < 0.01).

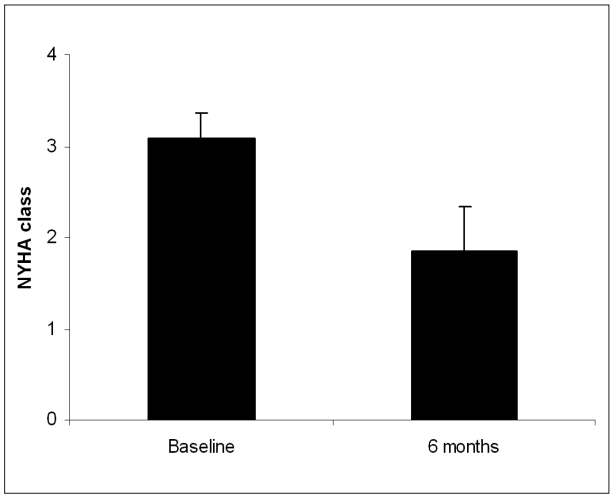

Figure 4 - Mean functional class (NYHA), before and after treatment. (Wilcoxon, P < 0.001).

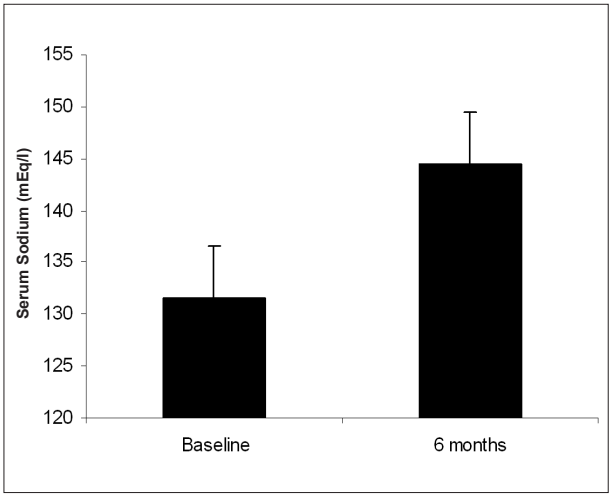

Figure 5 - Serum sodium concentration, before and 6 months after treatment. (Wilcoxon, P = 0.001).

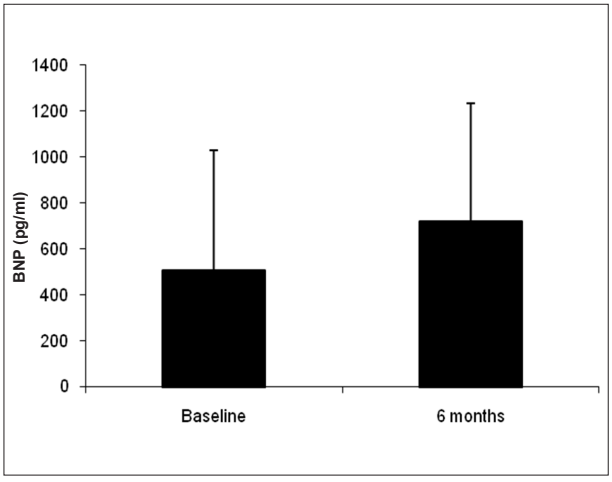

Figure 6 - MMP-2 levels, before and 6 months after BMC treatment. (Wilcoxon, P = 0.003).

No significant differences in plasma levels of the cytokines IL-1-beta (12 ± 14 to 13 ± 13 pg/ml, p = 0.54) and IL-6 (40 ± 74 to 28 ± 48 pg/ml, p = 0.21), as well as in TNF-alpha levels (3.4 ± 3.5 to 3.8 ± 4.2 pg/ml, p = 0.38) were observed.

Discussion

This study demonstrates that cell therapy carried out as an intracoronary injection of mononuclear cells derived from autologous bone marrow is feasible, safe, and appears to be effective in patients with advanced heart failure caused by Chagas' disease.

In these selected patients, with severe, stable and well treated heart failure, the procedure was well tolerated, and no arrhythmias, acute myocardial injury nor deterioration of cardiac performance was observed following the injection of cells into the coronary arteries. This finding is of great relevance, as previous studies using skeletal muscle stem

cells (satellite cells) have raised concerns regarding the development of arrhythmias in patients that underwent this kind of cell therapy[23,24].

Despite being on optimal treatment and multi-drug regimen in optimal doses, patients' functional capacity and quality of life remained severely impaired. However, there were consistent improvements in several of the evaluated parameters. Quality of life improved consistently, as evaluated by the Minnesota questionnaire, which reflects not only dyspnea parameters, but also emotional, psychological, economic, and professional aspects. The same was true regarding more objective variables, as distance covered during the six-minute walk test, which measures exercise capacity at sub-maximal levels. Consistent with the variations mentioned above, there was a significant improvement in functional capacity, as assessed by the NYHA classification.

LVEF increments were similar to those described in previous studies. Two recent systematic reviews and meta-analysis of intracoronary cell therapy found that this kind of therapy results in a modest, yet significant, increase in LVEF compared with control (3 to 4%)[25,26]. In the present study, mean LVEF increased 5%, corresponding to approximately 20% of relative increase, as LVEF of our patients was significantly lower. The issue of whether a small increase in LVEF is of clinical significance is an important question. Most of the life-saving interventions in cardiology do not substantially increase ejection fraction. The mechanisms behind the improvement in ventricular performance are a matter of debate. Recent data indicate that bone marrow-derived cells adopt mature hematopoietic characteristics[27,28]. Another proposed mechanism is that BMCT may exert paracrine antiapoptotic effects, inducing modifications in the immune milieu[29].

Considering the well-known difficulty in controlling hyponatremia with usual treatment, it is noteworthy the observation of normalization of serum sodium observed in this study[30,31]. The mechanism of correction appears to be independent from the level of vasopressin stimuli, considering that the vasopressin serum level remained elevated and remains to be evaluated in other experiments.

An interesting finding in our study was the behavior of BNP. At baseline, BNP level was markedly elevated, albeit when considering the severity of the heart failure, it would be expected to be even higher[32,33]. What was somewhat puzzling was the fact that despite the consistent clinical improvement observed by several parameters, its level did not decrease accordingly. There are some documentations demonstrating that occasionally, in very severely compromised ventricles, the BNP level might not rise as expected[34]. Mechanisms speculated in this regard may imply the possible exhaustion of cardiomyocytes to produce BNP. Along the same line of speculation, perhaps what we have seen is related to some degree of cardiomyocyte regeneration reestablishing its capacity to produce BNP, despite the partial clinical improvement. However, we cannot exclude the possibility of other cellular component regeneration contributing to this fact[35].

It's well known that inflammation plays a major role in the pathogenesis of Chagas' disease[8]. However, in our study, inflammatory biomarkers did not change in response to BMCT. This finding suggests that the cell therapy has no effect on the mechanisms of inflammation or else, other sources of hyperproduction of these substances are not affected by the utilized technique. It is interesting to register, however, that MMP-2 levels increased after BMCT, which might correlate with increased collagen turnover[36,37].

Considering the characteristics of the present study, a pioneer, phase 1 study, the efficacy of the procedure must be evaluated in a more appropriate design, which will ideally include a placebo-control group for comparison.

Nonetheless, we have demonstrated in human beings with severe advanced heart failure caused by Chagas' disease, the potential use of a new form of therapy that must be confirmed by other observations in the future. A multicenter randomized placebo-controlled study to test the effects of BMCT in Chagas' disease heart failure involving 300 patients is currently including patients[38].

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Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any post-graduation program.

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