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Bone Marrow Stem Cell Transplantation and Coronary Artery Bypass Grafting Surgery for Chronic Ischemic Myocardiopathy

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ABSTRACT

We studied 12 consecutive patients with chronic ischemic myocardiopathy treated with bone marrow adult stem cell (ASC) transplantation and coronary artery bypass grafting (CABG). The aim of the study was to evaluate functional class (New York Heart Association), wall motion score index (WMSI), and ejection fraction by echocardiography and to evaluate myocardial perfusion by single-photon emission computed tomography (SPECT). Follow-up evaluations were performed at 3, 6, and 12 months. The results revealed functional class improvement until 12 months, a progressive increase in the ejection fraction of 15% to 20% in the first 6 months, and a progressive increase in the WMSI by 35% to 45% in 12 months. Evaluation of the WMSI in the stem cell and CABG areas separately revealed a similar improvement in the first 3 months and a better progression in the CABG area. SPECT images revealed perfusion improvements in ischemic areas and no difference in fibrous tissue areas. These preliminary results show the safety of the method and its reproducibility. When performed concomitantly with CABG, bone marrow ASC transplantation may improve functional class, ejection fraction, WMSI, and myocardial perfusion. This study will be completed with all patients followed up for 12 months and compared with a control group.

INTRODUCTION

There are different types of stem cells. The basic difference is in the way they are obtained, as embryonic stem cells (ESC) or as adult stem cells (ASC) [Burt 2007]. ESC have the pluripotent capacity to generate any human cell type. In 1998, James Thomson (University of Wisconsin) isolated the first human ESC. The therapeutic potential of ESC is impressive and inspiring; however, their use carries religious, ethical, and moral problems. In addition, their use is associated with rejection, multiplication, and transdifferentiation under control. For these reasons, ESC studies are limited to the experimental area, with clinical applications of stem cells being limited to ASC [Burt 2007].

ASC can be found in bone marrow aspirates, skin, liver, the central nervous system, dental pulp, umbilical cord, adipose tissue, amniotic fluid, skeletal muscle, and other tissues. Hematopoietic ASC have been used for 3 decades in the treatment of leukemic diseases [Asahara 2000; Alves 2007; Burt 2007; Senegaglia 2007; Silva 2007; Zonari 2007]. Kocher et al published a study that showed that adult hematopoietic stem cells injected into areas of acute myocardial infarction cause vascular endothelial cell differentiation, prevent cardiomyocyte apoptosis, reduce ventricular remodeling, and increase cardiac function [Ryan 2000; Kocher 2001].

Cardiac myocytes do not multiply in culture, apparently owing to a resistance to restarting the cell cycle. This fact is one reason for the search for a new precursor muscle cell: the autologous myoblast [Gary 2003]. Menasché et al were the first to clinically use autologous myoblasts, which they injected into the heart of a 72-year-old woman with advanced heart failure. Five months after transplantation (with concomitant coronary artery bypass grafting [CABG]), these investigators found metabolic and contractile activity in the previously akinetic area, along with improvements in ejection fraction and functional class [Menasché 2003].

Brofman et al [2007] studied the effect of transplanting skeletal myoblasts in coculture with mesenchymal stem cells for the treatment of ischemic myocardiopathy. They observed great improvements in functional class and ventricular contraction. Strauer and Kornowski recently experimentally demonstrated the capacity of hematopoietic stem cells to transdifferentiate into vascular cells and myocyte-like cells [Strauer 2003]. On the other hand, the challenges have been greater than the successes. Several questions remain to be answered regarding the best cell type, the best mode of cell delivery, the time to a therapeutic effect, and the need to repeat the procedure.

The Stem Cells Research Center in Cardiology at the Clinics Hospital of Paraná Federal University began an investigation in July 2007 into the use bone marrow ASC concomitantly with CABG.

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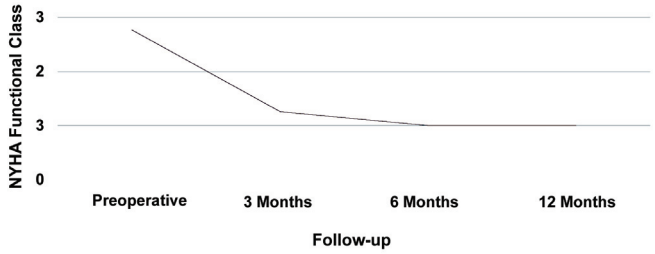

Figure 1. New York Heart Association (NYHA) functional class.

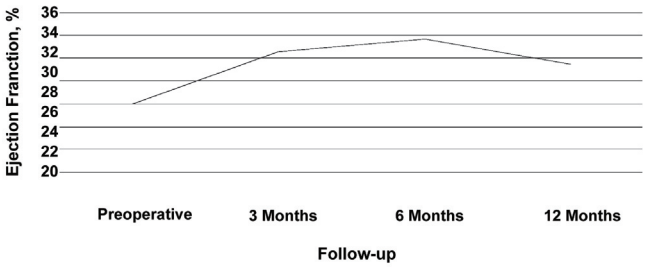

Figure 2. Left ventricular ejection fraction as measured by echocardiography.

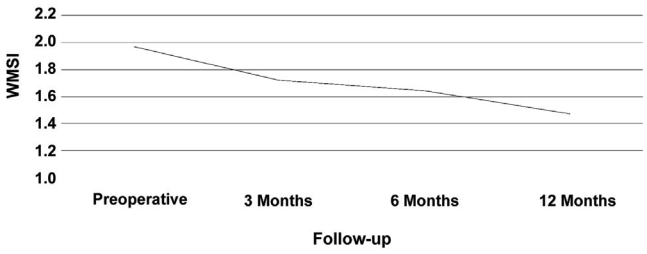

Figure 3. Wall motion score index (WMSI) as measured by echocardiography.

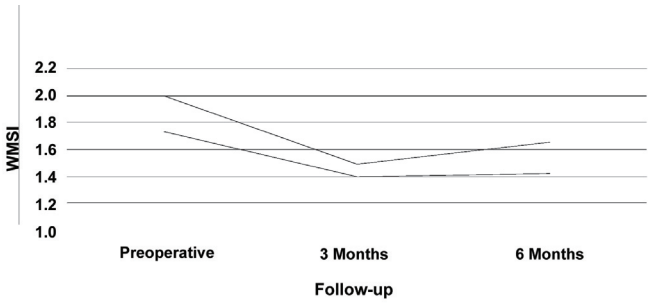

Figure 4. Wall motion score index (WMSI) in selected stem cell (CT) and CABG (RM) areas (P = .3).

OBJECTIVE

The objective of this study was to test the safety of the bone marrow stem cell transplantation procedure and to identify its benefits with respect to local contractility independent of the CABG areas.

MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committee of Paraná Federal University, and all patients freely consented to participate in this research. There were no conflicts of interest. This investigation was a prospective, open-blind, nonrandomized study. We present the preliminary results for 12 patients.

Inclusion criteria were an age ≥18 years, a surgical indication for treatment of coronary artery disease, an akinetic or hypokinetic left ventricle area for which CABG was impossible, and diagnosed heart failure (defined as an ejection fraction <35% by 2-dimensional echocardiography). Exclusion criteria were defined as follows: chronic rheumatologic disease, diagnosed malignancy, digestive tract bleeding, acute or chronic renal failure, blood transfusion within the previous 6 months, any disease that might decrease life expectancy, illicit drug use, and moderate or severe valve disease.

Bone marrow aspiration was carried out with local anesthesia and sedation. Bone marrow was obtained from the posterior superior bilateral iliac crest. A total of 100 mL was obtained by multiple 5-mL aspirations at different sites. The objective was to obtain ≥150 × 10^6 mononuclear cells.

The mononuclear bone marrow fraction was obtained by density-gradient separation with Ficoll-Hypaque (1.077 g/mL). Microbiologic control was performed. The trypan blue test was used to test for cellular viability. A total cell count and a $CD34^+$ immunophenotyping count were done before and after processing the samples.

The $CD34^+$ cells were counted with a FACSCalibur 4-color flow cytometer (BD Biosciences, San Jose, CA, USA). Fluorescein isothiocyanate–conjugated CD45, phycoerythrin-Cy5–conjugated CD45, and allophycocyanin-conjugated CD34 monoclonal antibodies were used to characterize hematopoietic stem cells [Stamm 2003].

Myocardial delivery of cells was carried out by direct injection after traditional CABG surgery was performed. Cells were injected into the beating heart so that the transition areas between the compromised and normal myocardium could be identified. Multiple 0.2-mL injections for a total of 8 mL of cell suspension were made in the transition area. Insulin-type needles were used.

To evaluate the effectiveness of this method, we used the following variables: a subjective evaluation of function according to the New York Heart Association (NYHA) classification, an objective ejection fraction measurement by a blinded 2-dimensional echocardiography evaluation, an objective and blinded evaluation of 17 segments of left ventricular wall motion by echocardiography to obtain wall motion score index (WMSI) values, and a blinded single-photon emission computed tomography (SPECT) evaluation of myocardial perfusion.

These evaluations were performed preoperatively and at the follow-ups at 3, 6, and 12 months postoperatively. Analysis of variance and the Student *t* test were used for statistical analyses. A *P* value <.05 was considered statistically significant.

RESULTS

We studied 12 consecutive patients with adequate preoperative evaluations and carried out follow-up evaluations at 3, 6, and 12 months. There were 3 deaths: 1 in-hospital death due to pulmonary sepsis in a patient with severe chronic obstructive pulmonary disease and 2 late deaths, possibly cardiac related (sudden death and acute myocardial infarction).

Examination of the NYHA functional class revealed a tendency for it to recover by 3 months, with a NYHA functional class maintained at approximately class I until the 12-month follow-up (Figure 1). The echocardiography evaluations revealed significant improvements in ejection fraction in the first 3 months, stability by 6 months, and a distinct regression by the 12-month follow-up (Figure 2). WMSI values progressively improved from the 3-month follow-up to the 12-month follow-up (Figure 3). Separating the wall segments that received stem cell transplants from those that underwent CABG revealed similar WMSI improvements in the first 3 months and better stability in the CABG areas (Figure 4).

The SPECT analysis showed perfusion normalization in the CABG areas. In the stem cell transplantation areas, there was perfusion recovery in the ischemic areas and no change in the fibroid tissue areas.

DISCUSSION

The plasticity of hematopoietic stem cells has recently been described, and their potential therapeutic use for tissue repair has been studied extensively. Different cell types are mobilized after myocardial injury for the repair of damaged tissue. Macrophages, monocytes, fibroblasts, neutrophils, and endothelial cells are directed to the infarcted area for repair and remodeling, which involve specific mechanisms involving cytokines, the extracellular matrix, and adhesion. Adult hematopoietic stem cell transplantation may become a therapeutic option for limiting the cardiac muscle fibers lost after a myocardial infarction, reducing or preventing ventricular remodeling, and treating heart failure [Hassink 2003; Lovell 2004].

Strauer et al from Dusseldorf, Germany, were the first to study the use of adult hematopoietic stem cells for myocardial regeneration in chronic ischemic cardiopathy. These investigators demonstrated reductions in the area of myocardial infarction, a 15% increase in the ejection fraction, and a 57% increase in the ventricular wall motion velocity. Regional consumption of oxygen and glucose was increased by 11% and 15%, respectively. In addition, Strauer et al demonstrated functional and metabolic recovery in chronic ischemic areas [Strauer 2002, 2003].

The possible mechanisms involved in the recovery of cardiac function when stem cells are used in patients with chronic coronary insufficiency are cytokine-induced cellular multiplication, increases in residual viable myocytes in periinfarct areas, stimulation of resident cardiac stem cells, and cell fusion of bone marrow stem cells with resident myocytes [Strauer 2005].

We treated 12 male patients in the present study. There were 3 deaths: 1 in-hospital death due to pulmonary sepsis in a patient with severe chronic obstructive pulmonary disease and 2 late deaths, possibly cardiac related (sudden death and acute myocardial infarction). Two patients underwent only bone marrow stem cell transplantation because of technical difficulties in performing CABG. These patients were excluded from the study. The complete analysis was made with 7 patients.

There was an improvement in NYHA functional class in all patients. Functional class improved from the third postoperative month and became stable until month 12 of follow-up. These results are similar to those of the Dusseldorf group and others [Perin 2003; Bartunek 2006].

The left ventricular ejection fraction increased by 15% in the first 3 months, was improved by 22% by 6 months, and returned to 15% after 12 months. Because of the small number of patients with 12 months of follow-up, we concluded that it is necessary to complete the evaluations for all of the patients to better define this curve.

The WMSI evaluation revealed progressive improvement from 3 months until completion of follow-up at 12 months. There was a 45% improvement in this index. This evaluation concerns all of the left ventricular segments, so this final result is attributable to both the stem cell and CABG areas. With this information, we separated the segments that received only stem cells and the segments that received only CABG. The results revealed better wall motion in both groups of segments at 3 months and distinct regression in the stem cell segments by 6 months.

The SPECT analysis revealed that ischemic areas become normal with both stem cells and CABG. The fibrous segments retained the same degree of perfusion with stem cell treatment.

These results agree with the results obtained in national and international research studies and provide the basis for us to continue our research. We recognize the need to complete the follow-up evaluations to 12 months for all of our patients and the need to incorporate the control group.

CONCLUSIONS

No new methodologic interference was observed in the patients' follow-ups. Our investigation of this group of patients revealed increases in NHYA functional class, ejection fraction, WMSI, and myocardial perfusion.

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