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Transplantation of Autologous Bone Marrow-Derived Cells into the Myocardium of Patients Undergoing Coronary Bypass

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ABSTRACT

Background: Animal studies suggest that cell transplantation, including bone marrow-derived cells, can ameliorate left ventricular remodeling following myocardial ischemia. Clinical evaluation of the potential benefits of this approach is limited by the lack of safety and feasibility studies. We have assessed the safety and feasibility of intramyocardial transplantation of autologous bone marrow-derived cells in patients undergoing coronary artery bypass graft (CABG) surgery.

Methods and Results: Between December 2001 and May 2002 7 patients, scheduled for CABG, consented to the trial. All had CABG using hypothermic cardiopulmonary bypass (CPB) and cold cardioplegic arrest. An average of 21×10^6 (8.6×10^6 to 35.1×10^6) nucleated cells, and 4.2×10^4 (2.5×10^4 to 8.1×10^4) CD34+ cells were injected into the anterior-lateral wall of the left ventricle, after discontinuation of cardiopulmonary bypass. The end points to assess safety included death, massive bleeding, electrocardiographic or biochemical evidence of myocardial infarction, ventricular dysrhythmia, myocardial perfusion, ventricular function, and the patients' functional status.

All patients recovered well without ventricular arrhythmia, bleeding, or other major peri-operative complications. The average intensive care unit (ICU) and hospital stay was 1 and 7 days, respectively. Repeat Technetium-99m myocardial perfusion stress imaging and echocardiography 6 weeks after surgery showed improvement in tissue perfusion, and an average improvement of left ventricular function of 13.5% ± 11.54% (the mean pre- and post-operative left ventricular EF were 32.5% ± 15.46% and 46% ± 18.55%, respectively). Twenty-four hours Holter monitoring showed no significant arrhythmia, 3 months post-operatively. All patients with narrow QRS complex showed no evidence of late potential, on signal-averaged electrocardiogram. At 4 to 9 months after surgery patients were in NYHA functional class "I".

Conclusions: This early clinical experience shows that autologous bone marrow-derived cell transplantation into myocardium is feasible and relatively safe. Further clinical trials to assess the role of cell transplantation for myocardial repair are required.

INTRODUCTION

Although CABG proved effective in preventing subsequent acute myocardial infarction, repair of previously damaged myocardium remains a major conundrum with no effective medical or surgical remedy. At present, regeneration of myocardial tissue from stem cells remains the most potentially viable option [Strauer 2002, Yau 2003]. Earlier experimental cell transplantation studies using skeletal satellite cells have shown that the grafted cells functionally integrate with and even augment the function of the recipient heart [Laham 2003, Yau 2003]. These findings have prompted the start of a number of clinical trials of intramyocardial myoblast transplantation in Europe and the United States [Laham 2003]. More recent studies, however, have shown that subsets of adult bone marrow cells can differentiate into endothelial cells, cardiomyocytes, and smooth-muscle cells in the infarct-bed after experimental myocardial infarction [Jackson 2001, Kocher 2001, Orlic 2001]. The differentiation of these cells is coupled with significant functional recovery, making adult bone marrow cells an ideal source of adult stem cells for transplantation therapy to prevent ventricular remodeling and to promote myocardial regeneration. We and others found that total bone marrow-derived cells contribute to new vessel formation in acutely ischemic tissues [Jackson 2001, Kocher 2001, Orlic 2001, El Oakley, 2002]. The safety and feasibility of bone marrow transplantation into human myocardium is a prerequisite before embarking on evaluating its potential clinical benefits. This report represents the first

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clinical study testing the feasibility and the safety of autologous bone marrow-derived cells transplantation into the human myocardium, as an adjunct to CABG for up to 9 months after surgery.

METHODS AND RESULTS

Between December 2001 and May 2002 seven patients signed an informed consent, previously approved by the Research and Ethics Committee of the National University Hospital, Singapore, to undergo this clinical trial. Their mean age was 57 years (46-71 years); they all had three-vessel disease with a mean left ventricular ejection fraction of 32.5% ± 15.46%. All patients had CABG using hypothermic cardiopulmonary bypass and anti-grade cold blood cardioplegic arrest (see Table). Before skin incision 5000 units of heparin were injected intravenously. Median sternotomy was performed and 20 to 50 mL of sternal bone marrow was aspirated for isolation of a buffy coat as described previously [Barnett 1998]; briefly, the bone marrow suspension was collected into 25 mL of RPMI with 10 IU/mL heparin in a plastic bag (Cobe Spectra Bone Marrow Processing Unit, Lakewood, CO, USA) and filtered (170 μM) into a transfer pack (Fenwal, Deerfield, IL, USA). The suspension was centrifuged at 4°C at 3200 rounds per minute for 15 minutes to obtain a 5 mL buffy coat, which was expressed into a sterile plastic bag, before transferring to the operating room. The average nucleated and CD34+ cell count was 21×10^6 (8.6×10^6 to 35.1×10^6), and 4.2×10^4 (2.5×10^4 to 8.1×10^4) per mL, respectively. After discontinuation of cardiopulmonary bypass five aliquots, 0.2 mL each, of the buffy coat were injected into the anteriolateral wall of the left ventricle at 2 cm intervals. Hemostasis was secured, the chest was closed using standard techniques, and the patients were transferred to ICU for close monitoring. In particular any form of ventricular dysrhythmia, excessive blood loss, electrocardiographic or biochemical evidence of myocardial infarction was recorded.

All patients recovered well without ventricular arrhythmia, or other major peri-operative complications. The average blood loss in the first 48 hours was 1153.43 mL. Three days after surgery one patient developed acute atrial fibrillation that was successfully treated with intravenous Amiodarone. None of the patients had electrocardiographic evidence of acute myocardial infarction. The mean CK-MB level 24 hours post-operatively was 5.33 ± 3.39 μg/L.

The average ICU and hospital stay was 1 and 7 days, respectively. Repeat Technetium-99m myocardial perfusion stress imaging, 6 weeks after surgery, showed improvement in tissue perfusion (see Figure). The enhanced tissue perfusion, which was noted in all cases, was associated with an average improvement in left ventricular ejection fraction was 13.5% ± 11.54% (the mean pre- and post-operative left ventricular EF was 32.5% ± 15.46% and 46% ± 18.55%, respectively). A 24-hour Holter monitoring showed no significant arrhythmia, 3 months post-operatively. Six patients with narrow QRS complex showed no evidence of late potential. One patient with prolonged QRS duration and conduction delay, left bundle branch block (LBBB), was excluded from the SA-ECG analysis, since SA-ECG has doubtful prognostic information in the presence of LBBB. All patients are in NYHA functional class "I", 4 to 12 months after the procedure.

Number of Grafts Performed and Their Related Territories

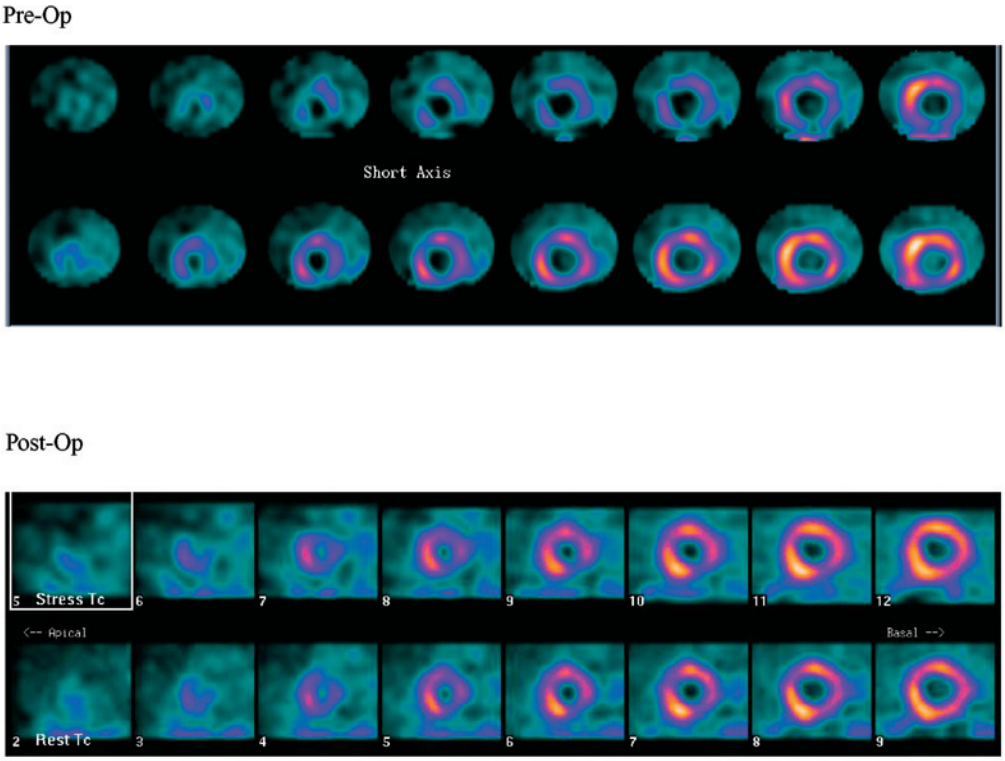
Patient Number/Name	Age	Grafts Performed
1. Narayani Sarada	71	LIMA to LAD + SVG to dRCA, OM1, and OM2
2. Cheng Get Sang	52	LIMA to LAD + SVG to PDA, OM, and D1
3. Tan Ah Chio	57	LIMA to LAD + SVG to dRCA and OM
4. Ng Poh Hiong	46	LIMA to LAD + RIMA to dRCA
5. Gee Kum Fatt	70	LIMA to LAD + SVG to dRCA
6. Yacob Bin Iman	52	LIMA to LAD + SVG to OM1 and PDA
7. Lim Kar Huat	55	LIMA to LAD + SVG to OM1

DISCUSSION

Bone marrow contains cells that seem to have retained the ability to differentiate into diverse tissues types including endothelial, skeletal, cardiac muscle cells, neural tissues, hepatocytes, etc [Strauer 2002]. The use of bone marrow-derived cells is a potential therapeutic option for myocardial injury [Jackson 2001, Kocher 2001, Orlic 2001]. Cell transplantation using subsets of bone marrow cells for myocardial repair has been demonstrated in animal models. Transplantation of Lin^- c-Kit^+ [Orlic 2001], Lin^- $CD34^-$/low c-Kit^+ Sca^+ [Jackson 2001], or human G-CSF mobilized Lin^- $CD34^+$ bone marrow cells [Kocher 2001] results in significant recovery of the heart muscle after acute coronary occlusion. Tissue repair, which is coupled with significant functional recovery, has been attributed to milieu-influenced differentiation of the stem cells into new blood vessels and/or cardiac myocytes.

These studies, together with a relatively easy access to sternal bone marrow during CABG, renders autologous transplantation of bone marrow cells into damaged myocardium a highly practical and potentially viable approach in facilitating cardiac regeneration after myocardial injury. Besides simplicity, this approach has the added advantage of using autologous bone marrow cells without additional invasive or expensive procedures. The more recent evidence of embryonic stem cell tendency to fuse with adult stem cells, making tetraploidy cell population, raises serious concern regarding the potential use of stem cells for organ repair [Terada 2002, Ying 2002]. The potential fusion of adult stem cells to the myocardial cells, if it indeed exists, is unlikely to have serious clinical consequences since the heart is exposed to circulating adult stem cells for its life span without an obvious clinical syndrome. Furthermore, the daughter cell arising from fusion of embryonic and adult stem cells [Terada 2002, Ying 2002] retains many characteristics of ES cells, suggesting that cell fusion may be an experimental aberration seen only in the presence of ES cells.

We have shown that transplantation of autologous bone marrow-derived cells into the myocardium is safe for up to 12 months duration. We have demonstrated the ease of harvesting, preparing, and injecting bone marrow cells within



A short-axis view of the pre- and post-operative vasodilator stress Technetium-99m myocardial perfusion scans of the first patient who had severe three-vessel disease and EF of 20%. The arrows denote the site of injection of 1 mL of bone marrow preparation containing 16.0×10^6 nucleated cells and 2.60×10^4 CD34+ cells per mL. The images show considerable improvement of myocardial perfusion including the site of cell transplantation (anterio-lateral wall of the left ventricle).

the physical and time constraints of coronary artery bypass operations. The improvement of patients' symptoms, and that of coronary perfusion and ejection fraction observed, can be attributed to CABG alone. The added benefits of cell transplantation can only be evaluated in a prospective randomized trial.

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