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Intramyocardial CD34$^+$ Cell Transplantation Combined with Off-Pump Coronary Artery Bypass Grafting

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ABSTRACT

This report describes a new therapeutic approach for severe ischemic heart disease, intramyocardial transplantation of autologous bone marrow–derived CD34$^+$ cells combined with off-pump coronary artery bypass grafting (CABG). CD34 is widely known as a cell surface antigen expressed on hematopoietic stem cells, and recent experimental studies have shown that CD34$^+$ cells include endothelial progenitor cells. We used the Isolex 300i magnetic cell selection system to separate CD34$^+$ cells from bone marrow cells. This report describes the first case treated with the combination of off-pump CABG and cell transplantation for therapeutic angiogenesis and myocardial regeneration. The transplantation of autologous bone marrow–derived CD34$^+$ cells improved perfusion of the ungraftable ischemic area.

INTRODUCTION

Despite the rapid progress in medical technology, the results of interventional cardiology and cardiac surgery for the treatment of severe ischemic heart disease accompanied by congestive heart failure remain unsatisfactory. Conventional coronary revascularization, such as percutaneus coronary angioplasty and coronary artery bypass grafting (CABG), provide little benefit, if any, for patients with diffuse coronary artery disease or calcified or narrow vessels. Incomplete revascularization and residual ischemia cause loss of cardiomyocytes and scar formation. Cardiomyocytes cannot be regenerated in the adult heart, so this process eventually leads to progressive ventricular remodeling and irreversible cardiac dysfunction. Therefore a new treatment strategy is required for patients with severe ischemic heart disease. Such a treatment would induce angiogenesis to restore the blood flow to the ischemic myocardium and myogenesis to repopulate contractile elements of the infarcted myocardium.

Bone marrow contains multipotent adult stem cells with a high capacity for differentiation. Recent progress in the area of regenerative medicine has made it possible to generate cardiomyocytes in vitro from bone marrow–derived cells [Makino 1999]. Several experimental studies have shown that bone marrow–derived cells can regenerate cardiomyocytes and blood vessels in damaged heart tissues, resulting in improvement of cardiac function [Orlic 2001]. This report describes a new therapeutic approach for severe ischemic heart disease, intramyocardial transplantation of autologous bone marrow–derived CD34$^+$ cells combined with off-pump CABG.

CASE REPORT

A 51-year-old woman presented refractory heart failure (New York Heart Association class III) and angina attack due to an extensive inferior myocardial infarction and anterolateral ischemia. Cardiac catheterization revealed total occlusion of the proximal right coronary artery (RCA) with distal filling by collaterals, severe stenosis of the left anterior descending coronary artery (LAD), and small size and diffuse narrowing of the left circumflex artery (LCX). Left ventriculography and echocardiography demonstrated inferior akinesis, diffuse hypokinesis, and a left ventricular ejection fraction of 46%. Preopretative stress adenosine triphosphate (ATP) single photon emission computed tomography (SPECT)-sestamibi perfusion scan showed stress-induced ischemia in the left ventricular anteroseptal and lateral areas, and fixed perfusion defects in the apex and inferior area. Off-pump CABG with the left internal thoracic artery (LITA) and the gastroepiploic artery (GEA) in situ was planned to revascularize the LAD and the RCA. Because there was no graftable coronary artery in the LCX area, the implantation of autologous bone marrow–derived CD34$^+$ cells was planned for therapeutic angiogenesis and myocardial regeneration. This procedure was approved by our institutional Ethics Committee, and after the patient had been given extensive information on the procedure, she gave her written informed consent.

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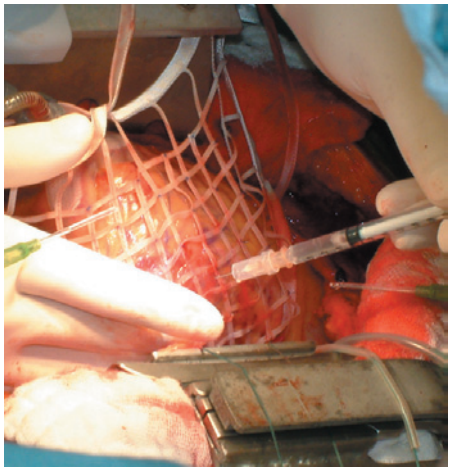

Figure 1. Implantation of autologous bone marrow–derived $CD34^+$ cells. The beating heart was stabilized with a heart net, then 2.1×10^7 $CD34^+$ cells in a 5-mL cell suspension were injected into 25 sites (0.2 mL to each site) with a 1 × 1–cm grid and a 26-gauge needle (6 mm deep).

With the patient under general anesthesia, collection of bone marrow cells from the iliac crest was performed in a standard fashion, followed by off-pump CABG procedure performed with a median sternotomy. The harvested bone marrow cells were diluted with RPMI 1640 (Gibco, Invitrogen, Carlsbad, CA, USA) containing heparin, and 750 mL was saved in a sterile pack from the bone marrow collection kit (Baxter, Chicago, IL, USA). The mononuclear cell fraction was sorted and concentrated to a final volume of 100 mL with a COBE Spectra Apheresis System (Gambro, Stockholm, Sweden). The total count of mononuclear cells was 3.73×10^9 cells. Next we used the Isolex 300i magnetic cell selection system (Nexell Therapeutics, Irvine, CA, USA) to separate $CD34^+$ cells from the fraction of mononuclear bone marrow cells. The final count of isolated $CD34^+$ cells was 2.1×10^7 cells. During cell purification, off-pump CABG was perfomed. The LITA was anastomosed to the LAD, and with sequential technique the GEA composite graft with the left radial artery was anastomosed to the RCA branches, the posterior descending coronary artery, and the atrioventricular nodal branch. When the off-pump CABG procedure was completed, implantation of $CD34^+$ cells into the entire LCX area was performed while the beating heart was stabilized with a heart net (Vital, Tokyo, Japan) (Figure 1). A 5-mL cell suspension containing 2.1×10^7 $CD34^+$ cells was injected into 25 sites (0.2 mL to each site) with a 1 × 1–cm grid and a 26-gauge needle (6 mm deep). There was no bleeding at the puncture sites. After implantation, ventricular pacing wires and drainage tubes were put in place, and the chest wall was closed.

The patient had an uneventful recovery. No changes or sustained ventricular or atrial arrhythmias were observed on electrocardiogram, nor was there any increase in the concentration of creatine kinase-MB, echographic evidence of pericardial effusion, or bleeding. After surgery, all symptoms of heart failure and angina attacks disappeared. Cardiac catheterization performed 32 days after surgery showed patent LITA and GEA grafts. Transthoracic echocardiography, performed at baseline and on day 28 after surgery, showed improvement of the left ventricular fractional shortening (from 25.8% to 32.1%) and a reduction in both the left ventricular endodiastolic (from 62 to 56 mm) and endosystolic (from 46 to 38 mm) dimensions. Stress ATP SPECT-sestamibi perfusion scans were performed at baseline and on day 28. The results showed an improvement in perfusion to the anteroseptal and inferior ventricular wall perfused with the LITA and GEA bypasses, and to the lateral ventricular wall implanted with $CD34^+$ cells. The fixed perfusion defect of the apex appeared not to have changed, however (Figure 2). Computed tomography examination performed 35 days after surgery showed no local calcification in the cell implantation area.

DISCUSSION

Most patients with severe ischemic heart disease who need cell therapy for angiogenesis and myogenesis generally suffer from advanced left ventricular dysfunction. In the treatment of such serious cases, conventional CABG procedure with cardiopulmonary bypass and cardiac arrest cause unfavorable outcome. Because the surgical procedure should be as noninvasive as possible, we performed both CABG and cell implantation on the beating heart without cardiopulmonary bypass. This case is the first to be treated with the combination of off-pump CABG and cell transplantation. During cell implantation, we used a heart net to stabilize the beating heart and used the meshes as a grid to mark the implantation sites. Because the meshes of the heart net compress the epicardium, they also helped to prevent bleeding from the cell implantation sites.

CD34 is widely known as a cell surface antigen expressed on hematopoietic stem cells. $CD34^+$ cells are used for the

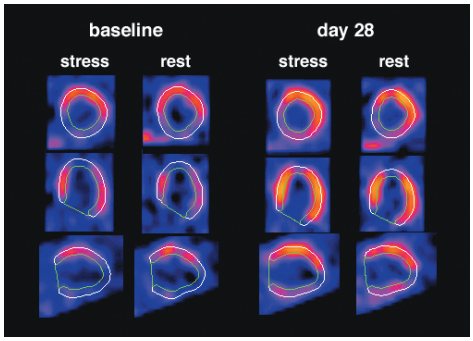

Figure 2. Baseline (pretreatment) and day 28 stress adenosine triphosphate single photon emission computed tomography–sestamibi perfusion scan results. Stress-induced ischemia is seen in the anteroseptal and lateral ventricular wall, and fixed perfusion defects in the apex and inferior area. On day 28 after surgery, perfusion was improved to the anteroseptal and inferior ventricular wall perfused with the left interior thoracic artery and gastroepiploic artery bypasses and to the lateral ventricular wall implanted with $CD34^+$ cells.

reconstitution of hematopoiesis after myeloablative therapy in the treatment of leukemia and cancer. Recent experimental studies have shown that CD34+ cells include endothelial progenitor cells, and Kocher et al [2001] have demonstrated that bone marrow–derived CD34+ cells induce angiogenesis of the ischemic myocardium, prevent ventricular remodeling, and improve cardiac function in animal models. In a human clinical setting, Stamm et al [2003] implanted autologous bone marrow–derived CD133+ cells in combination with conventional CABG. CD133 is also a cell surface marker expressed on hematopoietic stem cells and endothelial progenitor cells. We used autologous bone marrow–derived CD34+ cells purified with the Isolex 300i magnetic cell selection system. The cells selected with Isolex 300i, which are characterized by the absence of residual magnet particles on the cell surface, contained fewer apoptotic cells and consequently had greater functional capacity [Watts 2002].

It is very difficult to correctly evaluate the effect of implantation of autologous bone marrow–derived CD34+ cells into the LCX area, because there is some possibility that bypass grafting to the LAD and right coronary territories may increase blood supply to the LCX area through collateral circulation. Therefore, in order to conduct a strict evaluation, it is necessary to perform cell transplantation alone without any other coronary revascularization. But in the actual treatment of patients with severe ischemic heart disease, it is very rare that cell transplantation alone is required. It is certain that in the future cell transplantation therapy will come to be performed in combination with bypass surgery.

This study indicates that implantation of autologous bone marrow–derived CD34+ cells improves perfusion of the ungraftable ischemic area, but a longer follow-up is needed to thoroughly assess the effectiveness and safety of this treatment. Badorff et al [2003] have recently provided surprising proof that endothelial progenitor cells can transdifferentiate in vitro into functionally active cardiomyocytes. The therapeutic use of CD34+ cells may therefore aid cardiomyocyte regeneration in patients with severe ischemic heart disease, which is currently considered to be irreversible and untreatable.

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