

Clinical Labeling and Imaging of Transplanted CD133⁺/CD34⁺ Stem Cells in Patients with Ischemic Heart Disease

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ABSTRACT

The application of somatic stem cells has been shown to support the recovery of the myocardium in end-stage heart failure. A novel method for the intraoperative isolation and labeling of bone marrow-derived stem cells was established. After induction of general anesthesia, up to 400 mL of bone marrow were harvested from the posterior iliac crest and processed in the operating room under good manufacturing practice conditions by means of the automated cell-selection device Clini-MACS (Miltenyi Biotec). We subsequently injected autologous CD133⁺ and CD34⁺ stem cells in a pre-defined pattern around the laser channels in patients undergoing coronary artery bypass surgery and transmural laser procedures. Intraoperative isolation and labeling is an effective cell-separation tool for the future, considering that novel cell markers can be promising new candidates for cell therapy.

INTRODUCTION

Implantation of bone marrow-derived stem cells into the heart has been reported as a feasible method for myocardial regeneration after myocardial infarction [Orlic 2001; Pesce 2003]. Of particular interest in studies aimed at therapeutic angiogenesis are cells expressing CD133 and CD34 (cluster of differentiation) markers for primitive multipotent stem cells [Quirici 2001]. Previous data from patients who received CD133⁺ stem cells in combination with coronary artery bypass grafting (CABG) showed improved perfusion at sites of stem cell injection [Stamm 2003]. We have developed a novel method for intraoperative processing and clinical labeling of bone marrow-derived CD133⁺ and CD34⁺ stem cells that enables their therapeutic use for both elective and emergency revascularization. During the selection process, the stem cells also undergo clinically applicable labeling that allows magnetic resonance imaging (MRI) of the cells.

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METHODS

Preparation of Bone Marrow

All methods including aspiration of bone marrow cells and stem cell isolation were performed in the operation room according to good manufacturing practice standards (GMP). The Clini-MACS device (Miltenyi Biotec, Bergisch Gladbach, Germany) was used to process the bone marrow aspirate. GMP include practices required for adaptation for pharmaceutical manufacturing and quality control. As described below in further detail, the entire cell-separation and -enrichment procedure was done in a closed system.

All patients were briefed in detail about the cell transplantation procedure and the operation. Informed consent was obtained from all patients. All procedures conformed to our institutional guidelines and the guidelines of our local ethics committee. The bone marrow aspirate was taken from the iliac crest of the patients (n = 9) while they were under general anesthesia and immediately prior to CABG, as part of a clinical trial that combined CABG and transmural laser revascularization with injection of CD133⁺/CD34⁺ stem cells. The aspirate was taken with heparin-coated syringes, transferred into blood bags, and washed with the CliniMACS PBS/EDTA Buffer (phosphate-buffered saline and EDTA; Miltenyi Biotec) containing 0.5% human serum albumin.

Selection of CD133⁺ Cells and Stem Cell Labeling with CD34 Antibodies

The bone marrow cells were transferred into a centrifugation bag and washed with sterile CliniMACS PBS/EDTA Buffer (with 0.5% human serum albumin). After centrifugation, a Terumo Sterile Connecting Device (TSCD) (Terumo Deutschland, Eschborn, Germany) was used to connect the bag to a buffer waste bag. The supernatant was removed completely by means of a plasma extractor. The cell pellet was resuspended, and the volume was adjusted to 100 mL with CliniMACS PBS/EDTA Buffer containing 0.5% human serum albumin and 1.5 mg/mL clinical grade IgG (Sandoz globulin; CSL Behring, Hattersheim am Main, Germany).

The contents of 1 vial of CliniMACS CD133⁺ and/or CD34⁺ reagent (Miltenyi Biotec), which includes antibody and nanoparticle complexes, was injected into a bag containing bone marrow cells and incubated on an orbital shaker for 10 minutes at room temperature. After washing and processing, CD133⁺ cells were isolated with the CliniMACS cell separator [Ghodsizad 2004] (Video online).

Cell Analysis

The selected cell population was analyzed as previously described [Ghodsizad 2004]. We used a fluorescence-activated cell analyzer with highly specific monoclonal antibodies coupled to fluorescent dyes (Becton Dickinson, Heidelberg, Germany). The number of cells was registered by a cell counter (Sysmex Deutschland, Norderstedt, Germany). CD133⁺ and CD34⁺ progenitor cells were determined before and after the automated separation process. Aliquots were analyzed by fluorescence-activated cell sorting (Becton Dickinson) with R-phycoerythrin-conjugated CD133/2 (clone 293C3), fluorescein isothiocyanate-conjugated CD45, and allophycocyanin-conjugated CD34 antibodies (Miltenyi Biotec). Propidium iodide was used to determine cell viability. We used a modified protocol of the International Society for Hematology and Graft Engineering guidelines for determination of hematopoietic stem cells [Sutherland 1996]. We used the CD45 antibody and propidium iodide staining for appropriate identification of vital CD34⁺/CD133⁺ cells as a standard protocol [Sutherland 1996]. Intraoperatively, probes collected from the positive cell fraction were sent for routine screening for microbiological cultures. The postoperative results were the basis for the correct antibiotic therapy, if needed.

Cell Injection

The positive cell fraction was resuspended in 7 mL Clinimacs PBS/EDTA Buffer (with 0.5% human serum albumin), collected in tuberculin syringes, and distributed by transepimycardial injection. No more than 0.5 mL was injected at a site; injections were made at multiple sites in the desired part of the myocardium.

RESULTS

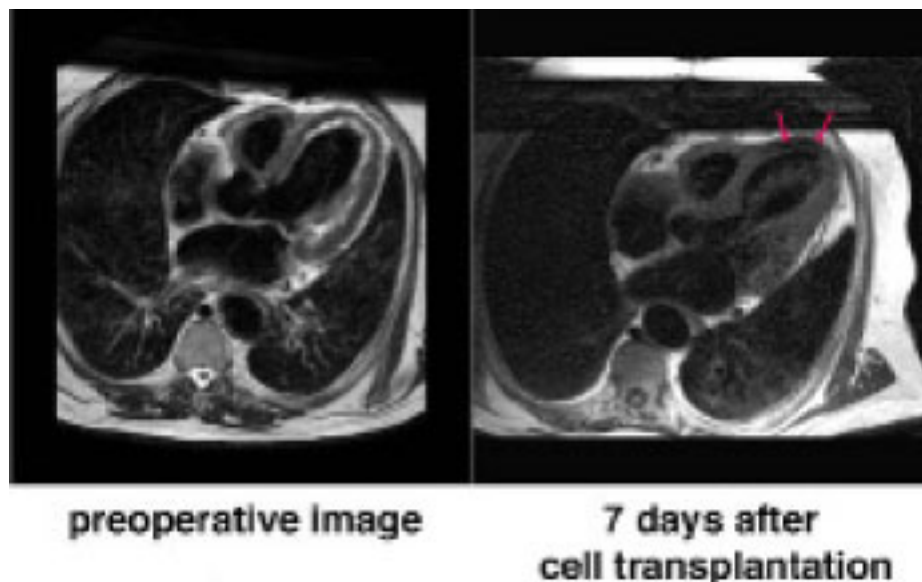
After the new procedure for the selection of CD133⁺/CD34⁺ stem cells, the filtered bone marrow had a normal morphology, and the microbiological screening showed no contamination. Using the intraoperative processing method, we were able to isolate up to 9.7×10^6 CD133⁺/CD34⁺ stem cells with a purity of >90%. Up to 9.7×10^6 CD34⁺/CD133⁺ cells were injected via the transepimycardial route. There were no adverse events. None of the patients presented with cardiac arrhythmia postoperatively. The labeled cells could be visualized with a 1.5-T MRI instrument (Siemens, Munich, Germany) at the site of the injection at 1 week postoperatively, but not later (Figure).

DISCUSSION

Different therapeutic strategies have been developed for patients with coronary heart disease not amenable to interventional or surgical treatment. Stem cell therapy, however, seems to be one of the promising future therapeutic options [Strauer 2001; Stamm 2003].

We demonstrated that intraoperative processing and labeling of bone marrow allows the selection of highly purified CD133⁺/CD34⁺ ethics committee-approved stem cells in <3 hours. Our technique provides a higher yield and a higher purity of bone marrow-derived CD133⁺ and CD34⁺ stem cells than the cell products isolated via Ficoll preparation and cell-selection techniques.

Our method allows surgeons to apply stem cells intraoperatively in emergency cases on the same day. In contrast to animal experiments, the potential is limited for collecting tissue samples from the sites of cell injection in the human



Cardiac magnetic resonance imaging showing the myocardium before (left) and after (right) transepimycardial stem cell transplantation.

myocardium. We have described intramyocardial detection of ferromagnetic bead-labeled bone marrow-derived cells via echocardiography [Ghodsizad 2006]. Kraitchman et al [2003] and Dick et al [2003] detected mesenchymal stem cells labeled with microparticles in swine myocardial infarction models via MRI [Kraitchman 2003] and magnetic resonance fluoroscopy [Dick 2003]. Our intraoperative magnetic selection method allowed us to label the injected cells through the use of CD34⁺ and CD133⁺ beads.

To our knowledge, this method is the first clinically applicable use of ferromagnetic beads for visualizing transplanted cells in the myocardium. Although there is no quantification or method available to determine how long these cells stay at the injection site as located by cardiac MRI, this method does have the ability to locate injected cells for a limited period of time postoperatively. As mentioned above, later imaging of the cells with cardiac MRI was not possible. The cells may have left the myocardial site of injection after a certain period of time. This finding is important for surgical or nonsurgical percutaneous transluminal catheter injections into the myocardium.

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