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Intramyocardial Implantation of CD133+ Stem Cells Improved Cardiac Function without Bypass Surgery

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ABSTRACT

Introduction. Cell transplantation for myocardial regeneration has been shown to have beneficial effects on cardiac function after myocardial infarction. Most clinical studies of intramyocardial cell transplantation were performed in combination with coronary artery bypass grafting (CABG). The contribution of implanted stem cells could yet not be clearly distinguished from the effect of the CABG surgery. Our current phase 1 clinical study has focused on the safety and feasibility of CD133+-enriched stem cell transplantation without CABG and its potential beneficial effect on cardiac function.

Method and Results. Ten patients with end-stage chronic ischemic cardiomyopathy (ejection fraction <22%) were enrolled in the study. Bone marrow (up to 380 mL) was harvested from the iliac crest. CD133+ cells were purified from bone marrow cells using the CliniMACS device with purities up to 99%. Autologous bone marrow CD133+ cells (1.5-9.7 × 10^6 cells) were injected into predefined regions. Cardiac functions prior to and 3, 6, and 9 months after cell transplantation were assessed by cardiac magnetic resonance imaging. Stem cell transplantation typically improved the heart function stage from New York Heart Association/Canadian Cardiovascular Society class III-IV to I-II. The mean preoperative and postoperative ventricular ejection fractions were 15.8 ± 5% and 24.8 ± 5%, respectively.

Conclusion. CD133+ injection into ischemic myocardium was feasible and safe. Stem cell transplantation alone improved cardiac function in all patients. This technique might hold promise as an alternative to medical management in patients with severe ischemic heart failure who are ineligible for conventional revascularization.

INTRODUCTION

Surgical or interventional revascularization of ischemic myocardium effectively treats angina, prevents myocardial infarction, and improves the function of still viable myocardium. However, the function of nonviable myocardium cannot be restored with current therapeutic means. Implantation of bone marrow–derived stem cells (BMSCs) into the heart has been reported to be a possible method for myocardial regeneration in patients with congestive heart disease [Hamano 2001; Assmus 2002; Strauer 2002; Ghodsizad 2006]. The transplantation of the CD133+ cells, a population of mononuclear cells, into human ischemic myocardium has demonstrated significant improvement of cardiac function [Stamm 2003; Klein 2004; Pompilio 2005]. Here we report on the application of CD133+ stem cells in 10 patients, who were treated with different dosages of injected cells without any concomitant revascularization.

METHODS

Patient Information

Between November 2003 and October 2005, 10 patients (8 male and 2 female) with ischemic cardiomyopathy were treated by injection of CD133+ BMSCs into the heart. Patients' ages ranged between 48 and 59 years (Table 1). Inclusion criteria were as follows: patients with ischemic cardiomyopathy, patients who could not be revascularized by conventional methods, and patients who had the presence of at least one localized area of akinetic left ventricular wall without paradoxical systolic movement. Patients with the following criteria were excluded from the study: history of myocardial infarction during the last 12 months, abnormal hemoglobin or abnormal count, and malfunction of platelets or leukocytes (eg, in malignancy or human immunodeficiency virus infection).

H. M. Klein, A. Ghodsizad, and A. Ruhparwar contributed equally to this paper.

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Table 1. Demographic Data of the Patients (N = 10)

Age, y	55 ± 8
Male sex	8
Hypertension	8
Diabetes	3
Hypercholesterolemia	6
Smoking	6
Previous myocardial infarction	8
Previous percutaneous coronary intervention	9
Previous coronary artery bypass grafting	6
Previous stroke	0
Peripheral vascular disease	5
Chronic renal failure	7
Multivessel disease	9
Mean ejection fraction at baseline	15.8 ± 5

The protocol was designed according to the laws and guidelines of the local human research ethics committee. The consent of the participants, which fulfilled the ethical and legal requirements, including the voluntary choice to participate and the comprehension of risks and possible outcomes, was obtained in all cases. When the patients were admitted, the examining physician evaluated their condition and cardiac function according to New York Heart Association (NYHA) and Canadian Cardiovascular Society (CCS) guidelines. Electrocardiography was performed to evaluate ventricular arrhythmia.

Cardiac Function

Magnetic resonance imaging (MRI) and echocardiography were used to evaluate the heart function. Hypoperfused, hypokinetic, and akinetic regions of the heart were thus identified for cell injection. Scar tissue was identified and excluded.

Cell Preparation

Under general anesthesia, bone marrow aspirate was drawn from the iliac crest using heparin-coated syringes. The bone marrow suspension was collected in blood bags and washed with EDTA–phosphate buffered saline containing 0.5% human serum albumin. The cell suspension was then filtered through a 100-µm filter to remove bone specula. The bone marrow aspirate was transferred between blood bags under GMP conditions. The CD133+ cells were processed and separated using the CliniMACS instrument (Miltenyi Biotec, Auburn, CA, USA) as described previously [Ghodsizad 2004]. Aliquots from the fresh bone marrow aspirates and from the injected cell fraction were collected to measure composition and purity by a fluorescence-activated cell sorter [Ghodsizad 2004]. Samples from the positive cell injection were collected immediately before intramyocardial injection and sent for microbiological screening to exclude contamination.

Cell Transplantation

Thoracotomy was performed in all 10 cases (lateral thoracotomy in 8 cases). Different numbers of CD133+ cells were isolated in each case. The cell suspension was aspirated in insulin syringes and injected transepicardially into 10 to 20 targeted points with a minimal distance of 1 cm between each.

RESULTS

The bone marrow cells were intact and normal in morphology after filtration of bone marrow. The mean purity of the CD133+ stem cells after purification and enrichment was 82.8% (range, 8.5%-99%). Microbiological screening showed no contamination prior to transplantation. Although the maximum yield of BMSCs was intended, different doses of CD133+ stem cells (1.5-9.7 million) could be isolated and were applied (Table 2). The mean procedure time was 3 hours 20 minutes. The mean time between the bone marrow aspiration and the cell injection was 2 hours 50 minutes. Patients were transferred to the intensive care unit and extubated after a mean time of 29 hours.

All patients were carefully selected; mean CCS class was 2.9 (±1.0) and mean NYHA class was 3.5 (±0.5) preoperatively (Table 3). One patient died on the third postoperative day. Electrocardiogram recordings showed no ventricular arrhythmia.

Table 2. Number of Injected Cells and the Change of Ejection Fraction (EF)*

Patient No.	BM	Injected Volume, mL	Purity, %	Injected cells, mill	CD133+ BMCs, mill	Change of EF, %
1	220	8	99	9.8	9.7	5
2	200	7	>90	10.1	9.1	140
3	220	4	78	11.4	8.9	93
4	240	5	90	10.2	9.2	38
5	220	6	88	9	8	53
6	300	10	>90	6.8	6.1	31
7	80	5	97	8.4	8.1	73
8	330	7	>90	5.4	4.9	35
9	380	4	8.5	18	1.5	41
10	200	7	97	7.7	7.5	0

*BM indicates bone marrow; BMCs, bone marrow cells.

Table 3. Comparison of Clinical Values at Baseline, 2 Months, 6 Months, and 9 Months*

	Preoperative	Postoperative		
		3 mo	6 mo	9 mo
Echocardiography				
LVEDD	79.2 ± 7	57.4 ± 3	59.4 ± 4	56.2 ± 5
Magnetic Resonance Imaging				
LVEF	15.8 ± 5	24.3 ± 6	24.1 ± 5.5	24.8 ± 5
LVEDV	210 ± 123.45	169 ± 79.5	162 ± 82	175 ± 70
Functional class				
NYHA	3.5 ± 05	1.3 ± 1	1.3 ± 1	1.6 ± 1
CCS	2.9 ± 1	1.2 ± 0.5	1.2 ± 1	1.2 ± 0.5

*LVEDD indicates left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; NYHA, New York Heart Association; CCS, Canadian Cardiovascular Society.

Cardiac MRI was performed 2 weeks prior to and 3, 6, and 9 months after cell transplantation (Figure 1). The mean left ventricular ejection fraction as determined by cardiac MRI was 15.8% (range, 10%-22%). The transplanted cells improved cardiac function up to 24.8% (range, 21%-27%) at 9 months after surgery. The mean CCS class was 1.2 (±0.5) and the mean NYHA class 1.6 (±1.0) postoperatively. The mean left ventricular end-diastolic volume, assessed by cardiac MRI, was 210 (±123.45) mL (range, 108-355 mL) prior to cell implantation and decreased to 175 (±70) mL (range, 96-255 mL) postoperatively at 9 months after cell transplantation (Figure 1). The left ventricular end-diastolic diameter, assessed by echocardiography, was 79.2 (±7) mm preoperatively and decreased to 56.2 (±5) mm after cell transplantation (Table 3). The mean preoperative end-systolic wall thickness was 5.7 (±0.7) mm and increased to 11.7 (±1.4) mm after stem cell therapy (Table 4).

DISCUSSION

Following the injection of CD133+ bone marrow cells into 10 patients with chronic ischemic heart failure, we observed a clear improvement of global left ventricular function as well as an improved contractility of myocardium in 8 patients. One patient died of low cardiac output syndrome. There were no procedure-related complications up to 9 months postoperatively, especially no new ventricular arrhythmia.

Early experimental studies have shown that the transplantation of autologous BMSCs into murine ischemic myocardium reduces the fibrotic scar area and improves cardiac function [Tomita 1999; Fuchs 2001; Orlic 2001; Toma 2002; Tang 2003]. The potential of the endothelial progenitor cells has been demonstrated in other publications [Kawamoto 2001; Kocher 2001]. Hamano et al have described the application of mononuclear cells as a method for inducing therapeutic angiogenesis in patients with ischemic heart disease [Hamano 2001]. The myoendothelial differentiation of CD133+ stem cells was presented by Pesce et al [2003]. Stamm et al have demonstrated that the transplantation of CD133+ stem cells can restore perfusion in ischemic myocardium and improve cardiac function [Stamm 2003]. Pompilio et al demonstrated a case of sole therapy with mobilized peripheral CD133+ stem cells for therapy of ischemic myocardium [Pompilio 2005]. Perin et al reported the improved exercise capacity of cardiac function after sole therapy with bone marrow–derived mononuclear stem cells for endstage ischemic heart disease [Perin 2004; Silva 2004].

To our knowledge, this is the first report about the clinical outcome after therapy of chronic myocardial ischemia with only CD133+ BMSCs. Our results provide the first clinical evidence that the application of CD133+ BMSCs

Table 4. Change of Wall Thickness after Cell Transplantation

	Mean	Minimum	Maximum
Preoperative end-systolic wall thickness, mm	5.7 ± 0.7	5	7
Postoperative end-systolic wall thickness, mm	11.7 ± 1.4	9	11
Improvement of wall thickness, %	207.2 ± 20.7	180	240

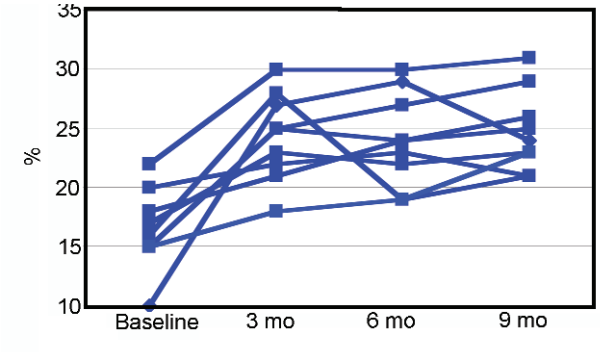

Figure 1. Ejection fraction at baseline, 3 months, 6 months, and 9 months.

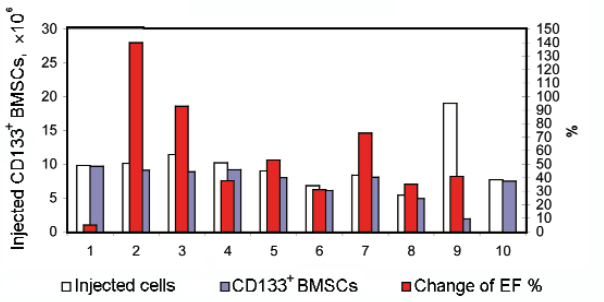

Figure 2. Summary of the total number of injected CD133+ bone marrow–derived stem cells (BMSCs) and the relative change of the ejection fraction (EF).

contributes to the improvement of cardiac function in patients with chronic ischemic heart failure. Other publications about the application of CD133+ stem cells described the combination of cardiac stem cell therapy and conventional revascularization methods [Stamm 2003]. A contributory effect of stem cells was assumed and the blood supply by collaterals was regarded as the important therapeutic account. We applied the highest number of CD133+ BMSCs harvested from human bone marrow for the transepicardial injection into ischemic myocardium [Stamm 2003]. The cell number we used may appear rather small compared with other studies, but it should be kept in mind that this is a purified population of cells with a high proliferation potential.

We were not intending to do a dose-expansion study, but the different number of implanted cells we had isolated showed no clear relation to the change of ejection fraction, excluding a dose dependence of stem cell effect on the change of the myocardial function. The other important finding is that not all patients respond in similar pattern to cell therapy (Figure 2).

Based on this data we conclude that transplantation of purified autologuous bone marrow stem cells as a monotherapy can be safely performed in humans. This technique looks highly encouraging as an alternative to the surgical management of ischemic heart failure in patients ineligible for conventional revascularization. Whether neoangiogenesis, neomyogenesis, and/or paracrine effects of the transplanted cells occur in the human situation remains unclear at this point. Interestingly, there was no overall correlation between the improvement of cardiac function and the total cell dose of mammalian cells as well as CD133+ BMSCs as a subfraction of these. One of our patients was treated with the highest number of mononuclear cells, but the lowest total number of CD133+ cells and showed a very good response (Figure 2). Further studies are required to determine the mechanism that starts to work after cell transplantation and the relevance of the purity of the injected cell population.

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