Intracoronary Injection of CD133-Positive Enriched Bone Marrow Progenitor Cells Promotes Cardiac Recovery After Recent Myocardial Infarction

Feasibility and Safety

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Background—Bone marrow CD133-positive (CD133+) cells possess high hematopoietic and angiogenic capacity. We tested the feasibility, safety, and functional effects of the use of enriched CD133+ progenitor cells after intracoronary administration in patients with recent myocardial infarction.

Methods and Results—Among 35 patients with acute myocardial infarction treated with stenting, 19 underwent intracoronary administration of CD133+ progenitor cells (12.6±2.2×10⁶ cells) 11.6±1.4 days later (group 1) and 16 did not (group 2). At 4 months, left ventricular ejection fraction increased significantly in group 1 (from 45.0±2.6% to 52.1±3.5%, P<0.05), but only tended to increase in case-matched group 2 patients (from 44.3±3.1% to 48.6±3.6%, P=NS). Likewise, left ventricular regional chordae shortening increased in group 1 (from 11.5±1.0% to 16.1±1.3%, P<0.05) but remained unchanged in group 2 patients (from 11.1±1.1% to 12.7±1.3%, P=NS). This was paralleled by reduction in the perfusion defect in group 1 (from 28.0±1.4% to 22.5±1.4%, P<0.05) and no change in group 2 (from 25.0±3.0% to 22.6±4.1%, P=NS). In group 1, two patients developed in-stent reocclusion, 7 developed in-stent restenosis, and 2 developed significant de novo lesion of the infarct-related artery. In group 2, four patients showed in-stent restenosis. In group 1 patients without reocclusion, glucose uptake shown by positron emission tomography with 18fluorodeoxyglucose in the infarct-related territory increased from 51.2±2.6% to 57.5±3.5% (P<0.05). No stem cell-related arrhythmias were noted, either clinically or during programmed stimulation studies at 4 months.

Conclusion—In patients with recent myocardial infarction, intracoronary administration of enriched CD133+ cells is feasible but was associated with increased incidence of coronary events. Nevertheless, it seems to be associated with improved left ventricular performance paralleled with increased myocardial perfusion and viability. (Circulation. 2005; 112[suppl 1]:I-178–I-183.)

Key Words: myocardial infarction ■ cells ■ restenosis

Although a number of clinical studies1–5 reported potential beneficial effects of intracoronary injections of mononuclear progenitors on cardiac regeneration, success in achieving this goal may be related to identification of the optimal cell type.1 Experimental studies indicate that progenitor cells with high hematopoietic and angiogenic activity are critical mediators of functional effects.6,7 Despite ongoing controversy with regard to cardiac transdifferentiation,4,8–10 c-kit–positive, lin-negative or CD34-positive cells contributed to cardiac repair by inducing angiogenesis, inhibiting apoptosis, and promoting myocyte recovery.6,7 In addition to the CD34 marker, CD133 marker, a prominin 5 transmembrane glycoprotein 1, is coexpressed in a substantial number of hematopoietic cells with potent hematopoietic and angiogenic capacity.11–14 In humans, proangiogenic effects of CD133-positive (CD133+) cells were suggested by improved myocardial perfusion after injections into the chronically infarcted myocardium at the time of the bypass surgery.15 Recent preliminary data suggest higher engraftment potential of selected hematopoietic cells as compared with mononuclear cells after intracoronary injection.16 Therefore, this phase I/II study tested the feasibility, safety, and functional effects of intracoronary administration of enriched autologous CD133+ bone marrow progenitors in patients with recent myocardial infarction.
Methods

Patients and Study Protocol
Thirty-five patients with recent myocardial infarction due to proximal occlusion of the epicardial coronary artery and treated with stenting were selected. Patients were eligible if they showed severe hypokinesia to akinesia of 2 adjacent segments on the baseline left ventricular (LV) echocardiogram performed 1 day after the infarction. Patients were divided into 2 groups: Group 1 consisted of 19 consecutive patients (18 with infarction in the left anterior descending artery, 1 in the angular branch) who underwent intracoronary injections of CD133+ cells between January, 2003, and May, 2004. Functional evaluations with LV angiography, electrophysiological study, technetium 99m sestamibi single photon emission computed tomography (MIBI SPECT), and positron emission tomography with 18fluorodeoxyglucose (FDG-PET) were performed before and 4 months after cell injection. Group 2 (15 left anterior descending artery, 1 right coronary artery) consisted of 16 patients presenting with acute myocardial infarction between January, 2001, and March, 2004, and matched for the infarction size and extent of regional dysfunction in whom follow-up LV angiograms and MIBI perfusion scintigrams were performed at baseline and between 4 to 6 months later. The study protocol was approved by the local ethical committee and informed consent was obtained in all patients.

CD133+ Cell Selection and Injections
Intracoronary cell injection was performed 11.6±1.4 days after the infarction. We collected >300 mL of bone marrow (BM) from the posterior iliac crest under general anesthesia. Mononuclear cells were isolated using 3 different methods, and CD133+ cells were selected from this fraction using ferrite-conjugated CD133 on the CliniMacs according to the manufacturer instructions (Miltenyi Biotech). For details, see Appendix I in the online-only Data Supplement. All cell preparations were suspended in 15 to 20 mL phosphate buffered saline/edetic acid (Miltenyi Biotech), including 1% of human serum albumin (albumine 20%; CAF/DCF). Purity of enriched CD133+ cells, determined as percentage of CD133+ cells in the final cell prepurate, ranged from 57% to 83%, with a total number from 1.5 to 33.6×10⁶ CD133+ cells. In the final cell suspension, 28% of the entire population of contaminating cells comprised CD14+ monocyte cells, 33% comprised CD56+ natural killer cells, and 19% comprised CD19+ B lymphocyte cells. Polymorphonuclear CD66b+ cells represented only 11% of the contaminating cells. No T cells, red cells, or platelet precursors were detected. CD133+ cells were injected into 10 hours after the BM puncture into the infarct-related (IR) artery using repetitive low-pressure balloon occlusions inside the stent as previously described.²⁻⁸ All cellular preparations used in the study were sterile.

Cardiac Catheterization
LV volumes and ejection fractions were calculated using the area-length method. Regional wall motion was analyzed using the centerline method. The ratio of LV systolic pressure to end-systolic volume was used as an index of myocardial contractility.²

MIBI SPECT and FDG-PET Scintigraphy
MIBI-SPECT scintigraphy was performed to estimate the extent of the perfusion defect by the bull’s eye technique. PET imaging was performed in subgroup of 11 treated patients at baseline and follow-up. The percentage FDG uptake was calculated as a marker of regional viability. Using the anatomy of the IR artery, a region of interest was generated, and absolute and relative indexes of metabolism were computed from this region. At least 2 remote segments per patient were defined as having normal perfusion, normal wall motion, and normal metabolism and were subducted by a normal coronary artery at angiography.

Safety Evaluation
To determine the safety of the CD133+ cell selection, standard laboratory tests, including C-reactive protein and troponin measurements, were performed before and 24 hours after cell administration. In addition, to assess the immunogenic potential of immunomagnetic CD133+ cell selection, human anti-mouse antibodies (HAMA) were determined before and 4 months after the cell injection using the commercially available kit (Immunomedics). Furthermore, all patients were continuously monitored until discharge and underwent 24-hour ECG monitoring at 2 weeks and 1 month after the procedure. Electrophysiological examination and quantitative coronary angiography were performed at baseline and at 4-month follow-up.

Data Analysis and Statistics
All invasive and noninvasive analyses were performed by operators blinded to all clinical and other functional data. Data are shown as mean±SEM. Paired t test and Mann-Whitney tests were used as appropriate. Fisher’s exact test was used for comparison of categorical variables. Statistical significance was set at P<0.05.

Results

Clinical Characteristics
As shown in Table 1, there were no differences in baseline characteristics between groups. There were also no differences in medical treatment with regard to statins, angiotensin-converting enzyme inhibitors, or β-blockers between groups (data not shown). Both groups were matched for the size and location of the infarction as evidenced by similar creatine kinase and troponin levels and number of Q waves on the ECG.

Feasibility and Safety of CD133+ Enriched Cell Injections
In group 1 patients, BM harvests were hemodynamically well tolerated by all patients. Twenty-four hours after intracoronary injections, a small, albeit a significant, increase in C-reactive protein was seen, with no change in troponin T noted (Table 2). In 5 randomly selected patients, HAMA antibodies remained negative 4 months after cell injection.

Adverse Events
In group 1, two patients required implantable cardioverter-defibrillator (ICD) implantation before the cell injections because of sustained ventricular tachycardia. At follow-up of 10±3 months, none experienced episodes of ventricular tachycardia or received appropriate shocks. One patient with an LV ejection fraction of 24% developed sustained ventricular tachycardia 2 days after the injection. No spontaneous sustained ventricular arrhythmias were noted in other patients. At a 4-month follow-up, electrophysiological exami-
In-stent restenosis was noted only in 4 group 2 patients and 2 showed a de novo significant lesion in the IR artery. Before and After Intracoronary Injection of CD133

LV function and no change in perfusion defect (Figure 2 and index. There was no significant change in global or regional showed a significant increase in LV end-diastolic volume in the IR territory was noted (Figure 3). Group 2 patients reocclusion of the artery, a significant increase in FDG uptake resting MIBI perfusion defect (Figure 3). In patients without chordae shortening was noted (Figure 2). This was associated with increased contractility and a significant decrease in the resting MIBI perfusion defect (Figure 3). In patients without reocclusion of the artery, a significant increase in FDG uptake in the IR territory was noted (Figure 3). Group 2 patients showed a significant increase in LV end-diastolic volume index. There was no significant change in global or regional LV function and no change in perfusion defect (Figure 2 and Figure 3).

**TABLE 2. Biochemistry, Coronary Events, and Arrhythmias Before and After Intracoronary Injection of CD133⁺ Cells (Group 1)**

<table>
<thead>
<tr>
<th></th>
<th>Before CD133⁺ Cells</th>
<th>Follow-Up</th>
</tr>
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<tbody>
<tr>
<td>Biochemistry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, ng/mL</td>
<td>13.5±3.2</td>
<td>20.4±3.6</td>
</tr>
<tr>
<td>TnT, IU/L</td>
<td>0.63±0.27</td>
<td>0.55±0.16</td>
</tr>
<tr>
<td>Pro BNP, pg/mL</td>
<td>1544±352</td>
<td>2310±528</td>
</tr>
<tr>
<td>HAMA antibodies</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Coronary events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-stent reocclusion</td>
<td>11 (2/19)*†</td>
<td>11 (2/19)*</td>
</tr>
<tr>
<td>In-stent restenosis</td>
<td>NA</td>
<td>37 (7/19)</td>
</tr>
<tr>
<td>De novo lesion</td>
<td>NA</td>
<td>11 (2/19)</td>
</tr>
<tr>
<td>Total coronary events</td>
<td>11 (2/19)</td>
<td>58 (11/19)</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous VT/VF</td>
<td>0/19</td>
<td>1/19‡</td>
</tr>
<tr>
<td>Inducible VT</td>
<td>2/19</td>
<td>1/19§</td>
</tr>
<tr>
<td>ICD Implantation</td>
<td>2/19</td>
<td>3/19</td>
</tr>
</tbody>
</table>

Values are mean±SEM, % (n/N), or n/N, as indicated. CRP indicates C-reactive protein; TnT, troponin T; BNP, brain natriuretic peptide; NA, not applicable; VT, ventricular tachycardia; VF, ventricular fibrillation.

*Includes a 31-year-old patient with protein C abnormality; asymptomatic acute in-stent thrombosis was detected before cell injections, and recurrent asymptomatic in-stent reocclusion was detected at 4-month follow-up.
†Includes a 45-year-old patient with symptomatic in-stent thrombosis before cell injection. No in-stent restenosis at follow-up.
‡Includes a 48-year-old patient with large anterior myocardial infarction and LV ejection fraction of 24%. VT storm occurred at day 2 after cell injection. After ICD implantation, the patient had an uncomplicated clinical course without life-threatening arrhythmias.
§Includes a 44-year-old patient with LV ejection fraction of 23%. At follow-up, there was inducible ventricular tachycardia with no spontaneous ventricular arrhythmias.
¶No firing detected in any patient with an ICD during follow-up of 10±3 months.

The present phase I/I study investigates the feasibility, safety, and potential of intracoronary administration of enriched CD133⁺ cells for functional recovery after recent myocardial infarction. The findings can be summarized as follows: (1) Injection of enriched CD133⁺ cells after recent myocardial infarction is feasible; (2) no increase in the incidence of ventricular arrhythmias was noted; (3) treated patients showed higher incidence of coronary events at 4-month follow-up; and (4) enriched CD133⁺ cells appear to contribute to functional recovery after myocardial infarction.

**Heterogeneous Versus CD133⁺ Enriched BM Progenitor Cells After Acute Myocardial Infarction**

Recent clinical studies²⁻⁵ suggested beneficial effects on cardiac repair using a heterogeneous population of mononuclear BM stem cells and led to the initiation of randomized trials. Before widespread clinical use of heterogeneous mononuclear progenitors can be pursued, however, the following factors should be considered. First, despite the potential advantage of using multiple progenitors, the function of many cell types from the mononuclear fraction after engraftment in the injured myocardium is unknown. Second, the number of progenitor cells with known function, such as mesenchymal cells or CD34⁺ and CD133⁺ cells, is very low. Third, multiple progenitors are likely to compete for engraftment during the transendothelial passage and may reduce the homing of cells with well-known effects. In this regard, recent preliminary data suggest an almost 7-fold higher homing capacity of enriched hematopoietic cells as compared with unfractonated mononuclear cells after intracoronary injections in patients with myocardial infarction.¹⁶ Finally, it remains unclear whether inflammatory progenitors, mostly present in the mononuclear fraction, are beneficial or whether they cloud the functional improvement. Thus, to optimize future clinical strategies, studies that address the effects of well-defined cell progenitors are of importance. Accordingly, in the present study, we tested the feasibility and safety of intracoronary administration of enriched CD133⁺ BM cells that are characterized by high pluripotent and angiogenic potential.¹¹⁻¹⁴

Despite being a phase I/I study, our findings suggest that intracoronary administration of enriched CD133⁺ cells is associated with improved LV function and decreased perfusion defect after recent myocardial infarction. Although we are presently unable to obtain pure CD133⁺ cell populations, the enriched CD133⁺ cells contained minimal contamination of natural killer cells, B cells, or monocytes. In addition, culture experiments of the final product failed to demonstrate the presence of attached mesenchymal cells (data not shown).

Therefore, we hypothesize that the functional effects observed in our study are mainly mediated by CD133⁺ cells and that these cells may represent the critical cell type needed for the functional recovery. Corroborating previous reports,³⁻⁵ our findings also suggest angiogenesis as a mechanism contributing to functional recovery. The concomitant increase in glucose uptake and myocardial contractility raises the intriguing possibility of the recovery of the contractile elements. Nevertheless, the cardiac differentiation of hematopoietic progenitors remains controversial,⁴⁻⁹ and improved

**Intracoronary CD133⁺ Enriched Cells and LV Function and Perfusion**

At baseline, functional parameters were similar between both groups (Figure 1 and Table 3). In the group 1 patients, a significant increase in LV ejection fraction and regional chordae shortening was noted (Figure 2). This was associated with increased contractility and a significant decrease in the resting MIBI perfusion defect (Figure 3). In patients without reocclusion of the artery, a significant increase in FDG uptake in the IR territory was noted (Figure 3). Group 2 patients showed a significant increase in LV end-diastolic volume index. There was no significant change in global or regional LV function and no change in perfusion defect (Figure 2 and Figure 3).
metabolism and viability may be related to myocyte recovery mediated by paracrine effects or cell fusion.\textsuperscript{9,10} The latter hypotheses are supported by the recent study of Agbulut et al.,\textsuperscript{17} who, despite the absence of changes in capillary density, observed a comparable degree of LV recovery after injection of CD133\textsuperscript{+}/H11001 cells or skeletal myoblasts in rats with semi-recent infarcts. Our study was not powered to address the effect of a possible preconditioning effect caused by repetitive balloon occlusion on recovery and the differences in functional changes between treated and nontreated patients. The magnitude of the observed functional changes, however, did not exceed the extent of the recovery reported in previous studies of mononuclear cells.\textsuperscript{2–5} Likewise, cell injections were performed later than in previous studies, and optimal timing for

Figure 1. LV angiogram (1A, 1B, 2A, and 2B), MIBI SPECT (1C and 2C), and FDG PET (1D and 2D) imaging before (1) and after (2) intracoronary injection of enriched CD133\textsuperscript{+} progenitor cells. An improvement in LV regional and global function with a reduction in the perfusion defect and an increase in FDG uptake are noted. The arrow indicates a significant in-stent restenosis at the control coronary angiogram (2E) versus baseline (1E).
cell injection has not been unequivocally defined. Thus, further randomized head to head comparison of enriched hematopoietic versus unfractionated mononuclear cell populations is required.

Feasibility and Safety of CD133+ Cell Injections

Functional recovery is likely to depend on the number of engrafted cells. In the study by Kocher at al, functional recovery was observed after intravenous injection of $10^6$ CD34+ cells. When taking into account only limited engraftment of $\sim$3% to 4%, one may extrapolate that at least $10^5$ to $2\times10^5$ cells/kg may be required for intracoronary injections. To obtain such numbers without culture expansion, a BM harvest of 350 to 400 mL is currently required. This harvest requires short general anesthesia, which was well tolerated in all patients. Also, a small rise in C-reactive protein levels may be related either to BM collection or repetitive occlusions during the cell injection. All preparations were performed under general medical panel conditions, and bacterial cultures tested negative. In addition, similar to previous studies on BM transplantation, serum analysis could not demonstrate HAMA antibodies. Additionally, ventricular arrhythmias were observed in 2 patients 2 days and 4 months after the injections. On the other hand, 2 patients with an ICD implanted before cell injections experienced no ICD activation during follow-up. These observations do not appear to support a causal relationship between cell injection and ventricular arrhythmias. Finally, the rate of in-stent restenosis or progression of atherosclerosis of the IR artery in this pilot study appears to be higher than in previous studies. The extent to which additional injury during repeated balloon inflations might have favored in-stent restenosis cannot be determined from this study. Drug-eluting stents were shown to significantly suppress in-stent restenosis in patients with acute myocardial infarction, and future studies are needed to test whether this strategy can avoid higher in-stent restenosis after intracoronary injection of enriched hematopoietic stem cells. Likewise, further studies are required to elucidate the effects of intracoronary injection of hematopoietic stem cells on the progression of coronary atherosclerosis.

Conclusions

In summary, present phase I/II study demonstrates that selection and intracoronary injection of enriched CD133+ cells in patients with recent myocardial infarction is feasible. Our data also suggest that CD133+ progenitors appear to contribute to functional recovery after recent myocardial infarction but do not support a causal relationship with ventricular arrhythmias. The extent to which additional injury during repeated balloon inflations might have favored in-stent restenosis cannot be determined from this study. Drug-eluting stents were shown to significantly suppress in-stent restenosis in patients with acute myocardial infarction, and future studies are needed to test whether this strategy can avoid higher in-stent restenosis after intracoronary injection of enriched hematopoietic stem cells. Likewise, further studies are required to elucidate the effects of intracoronary injection of hematopoietic stem cells on the progression of coronary atherosclerosis.

### Table 3. Effect of Intracoronary Enriched CD133+ Cells on LV Function and Perfusion

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=19)</th>
<th>Group 2 (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-Up</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>73±3</td>
<td>69±3</td>
</tr>
<tr>
<td>LVEDVI, mL/m</td>
<td>91±7</td>
<td>99±7</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>45.0±2.5</td>
<td>52.1±3.5*</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>111±4</td>
<td>123±4</td>
</tr>
<tr>
<td>LVSP/LVESVI, mm Hg/mL/m^-1</td>
<td>1.53±0.3</td>
<td>2.09±0.5*</td>
</tr>
<tr>
<td>Chordae short, %</td>
<td>11.5±1.0</td>
<td>16.1±1.3*</td>
</tr>
<tr>
<td>MIBI defect, %</td>
<td>28.0±1.1</td>
<td>22.5±1.4*</td>
</tr>
</tbody>
</table>

LVEDVI indicates LV end-diastolic volume index; LVEF, LV ejection fraction; LVSP, LV systolic pressure; LVESVI, LV end-systolic volume index. *P<0.05 vs baseline.

Figure 2. LV function and perfusion in treated patients and controls. The upper panel shows global and regional LV function. The lower panel shows changes in the ratio of peak LV systolic pressure and LV end-systolic volume index (LVSP/LVESVI) and LV end-diastolic volume index (LVEDVI). *P<0.05 versus baseline. Open bars indicate baseline; full bars indicate follow-up at 4 months.

Figure 3. MIBI perfusion defect (left panel) in patients treated with enriched CD133+ cells (empty bars) and in controls (filled bars). FDG uptake increases in the infarct-related (IR) territory at PET imaging in patients with CD133+ cells without re-occlusion of the IR artery at follow-up (right panel). *P<0.05 versus baseline.
Enriched CD133+ Cells and Cardiac Recovery

infarction. Thus, further randomized studies to address the safety and functional effects of enriched bone marrow hema-topoietic cells and their head to head comparison with unfractionated mononuclear cells are warranted.

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References

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