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Experimental Hematology

Experimental Hematology 35 (2007) 1884–1890

Evidence that intracoronary-injected CD133$^+$ peripheral blood progenitor cells home to the myocardium in chronic postinfarction heart failure

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(Received 27 April 2007; revised 17 July 2007; accepted 20 July 2007)

Objective. **To study the biodistribution of purified CD133$^+$ cells after intracoronary injection in patients with stable chronic postinfarction heart failure.**

Patients and Methods. **Patients with longstanding myocardial infarction (>12 months prior to inclusion) and with an accessible left coronary artery were eligible. CD133$^+$ cells were mobilized with granulocyte colony-stimulating factor and purified with a CliniMACS device. Cells were labeled with ^{111}Indium and injected through a balloon catheter in a coronary artery feeding the necrotic or viable infarct-related region of the left ventricle during a standard coronary catheterization procedure. The total body biodistribution of ^{111}Indium was studied with a dual-head gamma camera in combination with ^{99m}Technetium-sestaMIBI cardiac distribution analysis.**

Results. **The number of CD133$^+$ cells injected ranged between 5 and 10 × 10^6 cells (low dose, three patients) or between 18.5 and 50 × 10^6 cells (high dose, five patients). In the five patients receiving the higher cell doses, a clear residual radioactivity was observed at the level of the chronic injury at 2, 12, and up to 36 hours after injection. A detailed analysis in two patients showed 6.9% to 8.0% (after 2 hours) and 2.3% to 3.2% (after 12 hours) residual radioactivity at the heart. No adverse events were observed during the procedure and up to 3 months follow-up.**

Conclusions. **We demonstrate that CD133$^+$ progenitor cells are capable of homing to the postinfarction remodeling myocardium after intracoronary injections in patients with chronic postinfarction heart failure.** © **2007 ISEH - Society for Hematology and Stem Cells. Published by Elsevier Inc.**

Chronic postinfarction heart failure (CPIHF) is a progressive disease in which ventricular remodeling leads to global pump failure [1]. Ventricular remodeling is characterized by fibrosis, degradation of connective tissue, and myocardial cell loss and is a maladaptive response of the heart driven by the neurohormonal system. Pharmacological inhibition of the renin-angiotensin-aldosterone and the sympathetic nerve system has been demonstrated to influence this process positively, but cannot prevent progressive heart failure to occur.

Cell therapy may be an alternative strategy to prevent, decrease, or reverse remodeling in CPIHF. The bone marrow contains several types of progenitor cells that are candidates to restore vascularization or to transdifferentiate into functional cardiac cells, including endothelial cells, smooth muscle cells, and cardiomyocytes [2,3]. Several recent randomized clinical trials have shown that intracoronary (IC) delivery of bone marrow–derived cells, shortly after acute myocardial infarction (AMI), is safe and may improve ventricular function [4–8]. In uncontrolled phase II trials in patients with chronic heart failure due to ischemic cardiomyopathy, direct or guided intraventricular injection of bone marrow cell suspensions appeared to be safe, and resulted in improved left ventricular function

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0301-472X/07 $–see front matter.
doi: 10.1016/j.exphem.2007.07.012

[9–12]. In some recent trials, a functional improvement has also been observed after IC injection of marrow cells in patients with CPIHF [13,14]. The mechanisms of the functional improvement after cell therapy remain unclear. In numerous animal models, it was shown that bone marrow progenitor cells after IC or intracardiac delivery contribute mostly to angiogenesis and less to myocardial regeneration [15–19]. Therefore, cardiac microvascularization may be the principal target, and consequently, endothelial progenitor cells (EPC) may be the preferred cell type in the context of cell therapy for ischemic heart disease.

AMI is characterized by an acute local inflammatory reaction involving upregulation of chemokine receptors and adhesion molecules, thereby facilitating retention of cells involved in tissue repair, including progenitor cells [20–22]. These mechanisms are less pronounced or absent in CPIHF, so it is unclear whether bone marrow–derived cells are capable of homing to the myocardium in this situation. We addressed this question in the present study. Patients with CPIHF received an IC injection of peripheral blood derived $CD133^+$ progenitor cells labeled with ^{111}Indium (^{111}In). We determined the biodistribution of ^{111}In-labeled $CD133^+$ cells and focused on the remaining radioactivity at the level of the heart for up to 72 hours after injection.

Methods

Patients

Patients with CPIHF are included if they meet the following inclusion criteria: left ventricular rejection fraction (LVEF) $<50\%$ as measured by gated single photon emission tomography (SPECT) using ^{99m}Technetium (^{99m}Tc)-sestaMIBI; have at least one accessible major left coronary artery feeding the necrotic or viable infarct-related region of the left ventricle, based on previous therapeutic or diagnostic coronarography; and signed informed consent. Exclusion criteria were age older than 75 years, AMI within 12 months prior to inclusion, presence of left bundle branch block or ventricular pacemaker device, evidence of bone marrow failure, any serious comorbidity, life expectancy of fewer than 12 months. The study protocol was conducted in accordance with the Helsinki declaration and approved by the local institutional ethical committees of both participating centers.

Stem cell mobilization and collection procedure

Peripheral blood progenitor cells were mobilized with granulocyte colony-stimulating factor (G-CSF), with four (three patients) or eight (five patients) doses of 5 μg/kg administered subcutaneously with approximately 12 hours interval and starting two (three patients) or four (five patients) days prior to apheresis. Apheresis of circulating mononuclear cells was performed using a Cobe Spectra cell separator (Gambro BCT, Lakewood, CO, USA). Following standard procedures, a total of 5000 to 10000 mL blood was processed over a 60- to 120-minute period.

Stem cell purification and labeling

After collection, the cytapheresis product was kept overnight at 4°C. On the day of catheterization and within 24 hours after apheresis, the collected cytapheresis product was centrifuged using the COBE 2991 cell processor (Gambro BCT) to deplete blood platelets from the collected cell suspension. $CD133^+$ cells were isolated with the Miltenyi (Biotec GmbH, Bergisch Glad bach, Germany) immunomagnetic separation procedure, according to the manufacturer's instructions. In short, cells were incubated with magnetic microbeads, directly conjugated to CD133 antibodies, for 30 minutes at room temperature. After two wash cycles in phosphate-buffered saline-0.5% human albumin using the COBE 2991 device, the magnetically labeled cells were enriched on a positive selection column in the magnetic field of the CliniMACS device (Miltenyi). After recovery of the positively selected $CD133^+$ cells, samples were taken for immunophenotyping and microbiology testing. Before labeling, the cell suspension was washed with phosphate-buffered saline to remove all plasma and albumin. After centrifugation, the supernatant was replaced by freshly prepared ^{111}In-oxine solution in 0.1 M TRIS buffer pH 7.4 (Mallinckrodt, Tyco Healthcare, The Netherlands). For the first three patients and based on the assumptions that 100% of cells were to be labeled and that no tracer migration would occur over time after IC injection, our aim was to have maximum doses of 5×10^6 $CD133^+$ cells and 5 MBq injected (low-dose group). For the following five patients and based on the first biodistribution results, maximum doses were 50×10^6 $CD133^+$ cells and 50 MBq (high-dose group). For labeling, cells were incubated for 45–60 minutes with 37 MBq (low-dose group) and 111 MBq (high-dose group) ^{111}In. Cells were then washed three times with phosphate-buffered saline and resuspended in 2 mL 0.9% NaCl suitable for injection. Viability of cells after labeling was assessed by trypan blue staining. We also aimed at injecting a fixed dose of $5–10 \times 10^6$ unlabeled $CD133^+$ cells in each patient. Time span between labeling and IC delivery was approximately 1 hour and time span between harvest of stem cells and injection was less than 24 hours.

Intracoronary delivery of stem cells

Cell preparations were injected during a standard coronary catheterization procedure in a patent coronary artery, feeding the necrotic or viable infarct-related region of the left ventricle. For injection, a balloon catheter was used. After exact positioning, the balloon was inflated at low pressure for a maximum of 1 minute. During inflation, cells were injected using two to five fractional high-pressure infusions of 2 mL cell suspension.

During the procedure of vessel occlusion, 550 MBq ^{99m}Tc-sestaMIBI was injected intravenously in order to identify and delineate the myocardium and the target vascular territory. After coronarography, patients were hospitalized for observation for 24 hours. Electrocardiography and routine blood examinations (basic hematology and biochemistry), including troponin levels, were performed on the day after the procedure. Patients had a clinical (including electrocardiography) and blood examination after 1 week and monthly thereafter.

Biodistribution of ^{111}In-labeled $CD133^+$ cells

Imaging was performed with a dual head gamma camera (MultiSPECT 2; Siemens, Inc., Hoffman Estates, IL, USA) equipped with medium energy collimators. Planar whole body acquisitions were acquired at 2, 12, 36, and 60 hours after stem cell transplantation, scan speed was set at 15 cm/minute. Additional SPECT images of the thorax were acquired at 2 and 12 hours

postadministration (90 projections, 15 seconds/stop, 45 stops over 360 degrees, matrix size 64 × 64). At 2 hours, a dual-isotope imaging protocol was used with photopeaks set at 140 keV for ^{99m}Tc and 171/245 keV for ^{111}In. At 12 hours, the energy window was set for ^{111}In only.

From the planar whole body images, biodistribution data were obtained by drawing regions of interest around the heart, liver, and spleen on the anterior views at the different time points after injection. Organ activities were expressed as a percentage of the total counts in the first whole body scan, which corresponds to the injected dose. Activities were corrected for isotopic decay.

Functional analysis

Cardiac magnetic resonance imaging (MRI) was used to evaluate LVEF at baseline and at 6 months after IC injection of the CD133$^+$ cells. Cardiac MRI was performed on a 1.5-Tesla Siemens Symphony scan (center A, four patients) or a 1.5-Tesla GE HD Excite scanner (center B, four patients). A detailed functional analysis was not a primary endpoint of this study and we did not systematically measure changes in segmental wall motion or thickness. The LVEF was calculated based on short axis true FISP images using Argus software (center A) or Fiesta images using Massanalysis (center B). The epi- and endocardial contours of the left ventricle were drawn manually by the radiologist and, based on these regions of interest, the LVEF was calculated by the software.

Results

Patient characteristics

Eight patients were included, six were male and two female, median age was 53 years (range, 50–72 years). Myocardial infarction occurred at a median of 48 months (range, 12–240 months) prior to inclusion. Baseline LVEFs (sesta-MIBI) were 14%, 17%, 20% 22%, 30%, 37%, 39%, and 47%. All patients had given written informed consent prior to inclusion in the study.

Stem cell collection, purification, and labeling

Numbers of CD133$^+$ cells collected and purified, the number of labeled CD133$^+$ cells injected and the corresponding radioactivity and the numbers of unlabeled CD133$^+$ cells injected are shown in Table 1. Median purity of CD133$^+$ cells before labeling was 92% (range, 79–96%). Median efficiency of ^{111}In-oxine labeling was 51% (range, 34–71%) and the viability of the cells after labeling averaged a median of 88% (range, 73–97%).

Adverse events

No unexpected or serious adverse events were observed during the stem cell mobilization and apheresis procedures or were related to the coronary catheterization procedure. There was one case of sustained ventricular tachyarrhythmia requiring cardiac pacing at 4 months and one progressive coronary stenosis requiring angioplasty at 3 months after procedure. Because they occurred at a distant time point from the catheterization, a relationship of these events with the stem cell injection was considered unlikely.

Table 1. CD133$^+$ cells ($\times 10^6$) collected and injected

UPN	No. of CD133$^+$ cells collected	No. of CD133$^+$ cells after purification	No. of labeled CD133$^+$ cells injected (MBq)	No. of unlabeled CD133$^+$ cells injected
1	21	17	1 (5.5)	4
2	30	22	2 (5.3)	8
3	90	47	5 (5.5)	5
4	343	320	25 (46.2)	9
5	348	229	40 (25.7)	10
6	145	98	27 (25.2)	10
7	62	55	14 (33.3)	4.5
8	158	135	22 (27.1)	10

UPN = unique patient number.

Biodistribution of ^{111}In-labeled injected CD133$^+$ cells

In the first three patients receiving a relatively "low dose" of radiolabeled cells, faint ^{111}In activity was observed in the heart region for the first 2 hours after cell administration. In all five patients receiving higher doses, a clear residual radioactivity at the level of the heart was detectable at 2, 12, and 36 hours after injection (Fig. 1). Detailed regions of interest analysis in two patients showed residual radioactivity at the heart of 6.9% to 8.0% after 2 hours and 2.3% to 3.2% after 12 hours (decay-corrected and expressed as a percentage of total body initial radioactivity). Liver and spleen uptake estimated, respectively, 23.1% to 26.8% and 3.1% to 3.7% after 2 hours and 23.8% to 28.3% and 3.5% to 3.8% after 12 hours (Fig. 2). The remaining fraction of labeled CD133$^+$ cells were evenly distributed over the other parts of the body, resulting in a homogeneous background activity, making identification of organs other than liver and spleen impossible. A fraction of free ^{111}In is excreted in the urine. Fused ^{99m}Tc-sestaMIBI/^{111}In SPECT images showed that the hypoperfused infarction zone—as delineated by ^{99m}Tc-sestaMIBI—was clearly visualized after IC injection of ^{111}In-labeled CD133$^+$ peripheral blood progenitor cells (Fig. 3). Regional distribution of transplanted cells within the target zone remained unchanged over time.

Functional analysis

MRI showed a significant improvement as compared to baseline in the overall LVEF (MRI) (increased from 30% to 41%) in only one of the seven patients evaluated. No significant improvements were observed in the other patients. Mean LVEF for these seven patients was 23.7% ± 13.5% at baseline, which was not different ($p = 0.85$ using Wilcoxon signed rank test) from 24.0% ± 15.0% at 6 months after IC injection.

Discussion

Our primary objective was to study the biodistribution of CD133$^+$ cells after IC injection in patients with

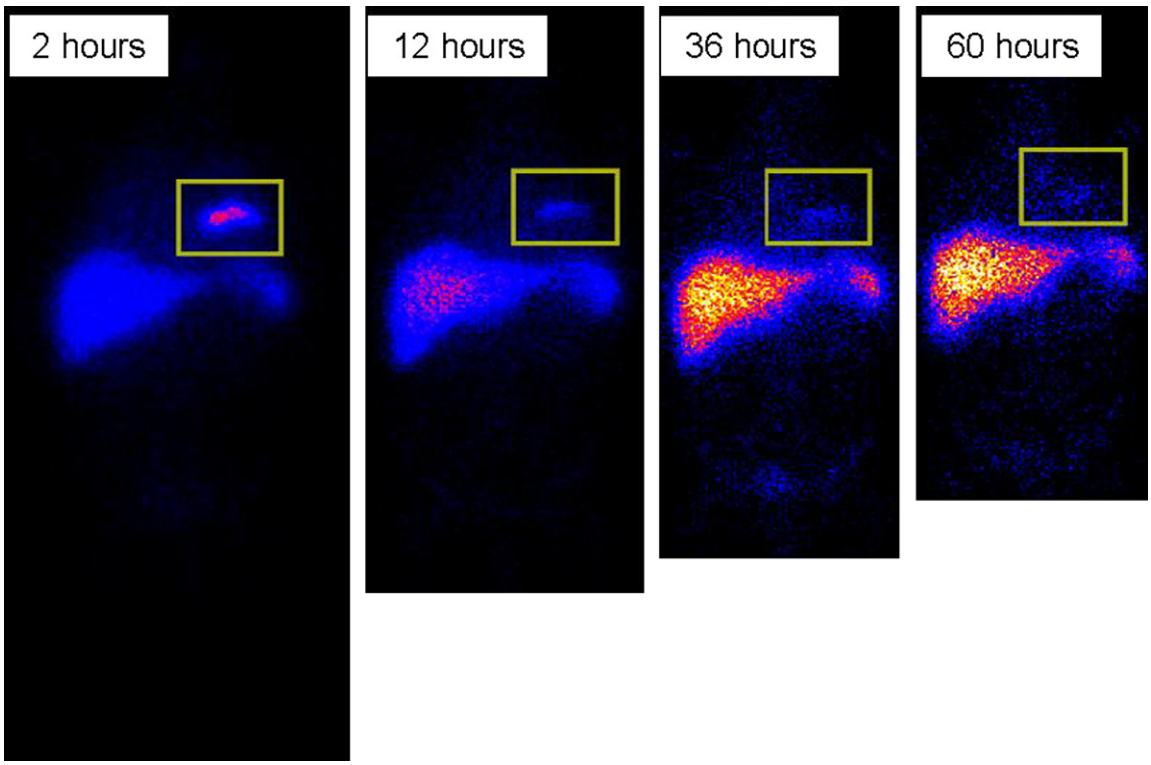
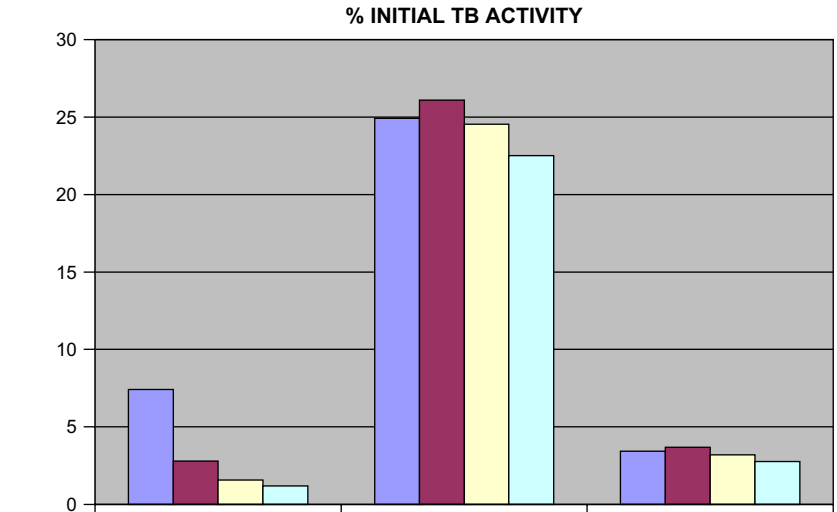


Figure 1. Anterior whole body scans acquired at 2, 12, 32, and 60 hours post intracoronary injection, showing the distribution of ^{111}Indium-labeled CD133$^+$ cells in a patient injected with 25×10^6 labeled cells. Residual activity at the level of the heart is indicated by rectangles and clearly detectable for up to 36 hours after injection.

longstanding CPIHF following AMI. We used purified CD133$^+$ cells for IC injection because the CD133 surface antigen defines a subset of CD34$^+$ hematopoietic stem cells enriched for EPC [23]. EPC are involved in vasculogenesis and angiogenesis and, from several preclinical studies, it appeared that revascularization is the main mechanism leading to improved cardiac function after the injection of bone marrow–derived cells [24]. Revascularization is essential for paracrine signaling processes, which are important in preventing remodeling of ischemic myocardium [25]. Furthermore, CD133$^+$ cells have already been delivered to patients with CPIHF by direct intracardial injection, which resulted in improved cardiac function [11]. We labeled CD133$^+$ cells with ^{111}In-oxine, which is a neutral and lipid-soluble complex that penetrates the cell membrane, and is widely accepted for radiolabeling different cell types in clinical practice. ^{111}In-oxine emits gamma rays suitable for imaging (172 keV and 246 keV) and because of its half-life of 67.2 hours (2.8 days) it allows monitoring of cell distribution over several days.

For IC injection, we used the stop-flow technique to prevent backflow of cells and prolong contact time to allow better migration of the cells to the myocardium. However, this technique could also create local downstream ischemia, thus rendering the local microenvironment more receptive to marrow cell homing. Using SPECT data of ^{111}In-labeled CD133$^+$ cells fused with ^{99m}Tc-sestaMIBI distribution, we could clearly demonstrate that, in the experimental conditions used, the labeled cells were localized in the infarct zone as depicted by the perfusion tracer and remained at this site over time. After IC injection, 7% to 8% of CD133$^+$ cells are retained in the postinfarction zones after 2 hours and at least 2% to 3% remain in situ after 12 to 24 hours. Decrease in signal intensity in the myocardium can be explained to some extent by cell lysis but, more importantly, by continuous ^{111}In efflux from intact cells, as prescribed previously [26]. For this reason, we think that the signal measured at the level of the heart is an underestimation of the true percentage of viable CD133$^+$ cells remaining in the target zone. The activity measured in the liver is due to sequestration of labeled cells as well as to accumulation of free ^{111}In in the reticuloendothelial system. This might explain why the activity in liver and spleen coincides with the physical decay of ^{111}In.

Patterns of biodistribution of the labeled CD133$^+$ cells observed were very similar in the five patients receiving the higher cell dose. Therefore, despite the small number of patients studied, we are quite confident that our observation indicates that CD133$^+$ cells have the capacity to penetrate and home into chronic postinfarction zones after CI. This is an important observation because homing is a prerequisite condition prior to other possible cellular effects aiming at functional improvement. As to the mechanisms of this phenomenon, we can only speculate. In



	HEART	LIVER	SPLEEN
2 hrs	7.43	24.92	3.42
12 hrs	2.79	26.09	3.69
36 hrs	1.58	24.53	3.20
60 hrs	1.20	22.52	2.77

Figure 2. Biodistribution of ^{111}Indium as measured by residual radioactivity in different organs at several time points after injected and represented as percentage of total initial body radioactivity (TB). The percentage shown is the average between the values obtained in two patients analyzed in detail and receiving the higher cell doses.

patients with AMI, the biodistribution of IC-delivered bone marrow cells has been studied with positron emission tomography scan after labeling of the cells with ^{18}F-fluoro-deoxy-glucose. At 50 to 75 minutes after injection, significant uptake at the level of the heart was observed. Retention of a CD34-enriched fraction was higher (14–39%) compared to unselected bone marrow cells (1.3–2.6%) [27]. The acute ischemia and inflammation in AMI increase the level of stromal–derived factor-1 (SDF-1), induce caveolin expression in the bone marrow microenvironment, increase sequestration of CXCR4 on marrow progenitor cells, all leading to mobilization of EPC to the site of injury [20,28]. Acute inflammation is absent in CPIHF, but several animal models have demonstrated that, also in subacute myocardial infarction, enhanced expression of SDF-1 in postinfarction zones is crucial for the homing and contribution to cardiac repair of G-CSF–mobilized peripheral blood stem cells [20,28,29]. We used CD34$^+$CD133$^+$ cells mobilized with G-CSF, which express high levels of the CXCR4 chemokine receptor [30,31] and, therefore, should have increased homing capacity to sites with increased SDF-1 expression.

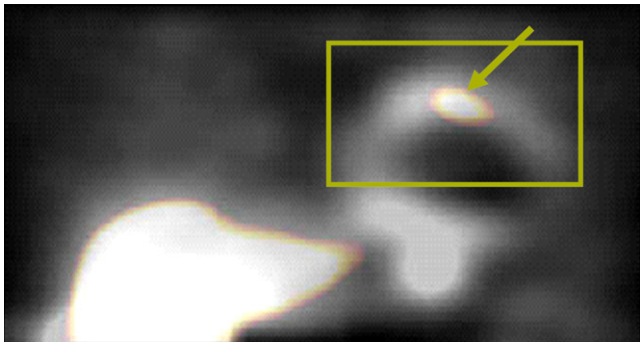


Figure 3. Coronal slice at the level of the heart. Image obtained 12 hours after injection of ^{111}Indium-labeled CD133$^+$ cells demonstrating a clear signal at the level of the heart (arrow) as well as accumulation in the liver. The data are merged with images of the ^{99m}Technetium-sestaMIBI distribution in the same individual acquired 2 hours after intravenous administration and depicted in gray (rectangle).

Despite the fact that homing was clearly demonstrated in our study, we were unable to show significant improvements in LVEF at 6 months after injection in six of the seven patients studied. Clinical experience on use of intracoronary injection of progenitor cells in patients with CPIHF after myocardial infarction is limited thus far. In a recently published randomized trial, small but significant functional improvements were observed after injection of bone marrow cells. No beneficial effect could be detected after injection of in vitro expanded peripheral blood progenitor cells [13]. In another randomized trial, patients with myocardial infarction having occurred at a median of 17 months prior to injection were included. G-CSF–mobilized peripheral blood mononuclear cells, including a median of approximately 30×10^6 CD133$^+$ cells, were injected, comparable to our study. The treatment did not result in an average functional benefit [32]. Based on our

observation, the limited functional benefit of IC injection of marrow-derived progenitor cells is not due to an intrinsic homing defect of G-CSF–mobilized $CD133^+$ progenitors to chronic postinfarction myocardium. However, we also demonstrate that only a small fraction ($<10\%$) of the injected cells, which are supposed to be functionally active, is retained for a prolonged period of time. This amount of cells is comparable to the white blood cell content of 1 to 2 mL blood in our study and others [32]. Such a low amount of cells is unlikely to induce remarkable functional effects. Use of higher cell doses or repeated injections as well as the number of injected cells in relationship to the size of the infarcted zone must be addressed in future studies. In addition, the functional capacity of EPC prior to injection may be enhanced by reconditioning or engineering. In a mouse model, exposure of the stem cells to hypoxia prior to injection increased their capacity to rescue cardiomyocytes from apoptosis [25].

In summary, our study shows that IC-injected $CD133^+$ progenitor cells are capable of homing to the myocardium in CPIHF. This observation offers a platform for other preclinical and clinical trials aimed at improving functional benefits of stem cell therapy in this setting.

Acknowledgments

The study was performed with financial support from the Fonds Willy Gepts UZ Brussel.

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