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# Myocardial Homing of Nonmobilized Peripheral-Blood CD34$^+$ Cells After Intracoronary Injection

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## ABSTRACT

**Granulocyte–colony-stimulating factor administered for autologous hematopoietic stem cell isolation from blood may favor restenosis in patients implanted after acute myocardial infarction (AMI). We therefore tested the isolation of peripheral-blood CD34$^+$ cells without mobilization in six patients with AMI. After large-volume cytapheresis and positive CD34$^+$ cell selection, 3.6 to 27.6 million CD34$^+$ cells were obtained. We performed intracoronary implantation of these cells and recorded no restenosis or arrhythmia. We used positron emission tomography (PET) to assess myocardial-labeled CD34$^+$ cell homing, which accounted for 5.5% of injected cells 1 hour after implantation. In conclusion, large amounts of CD34$^+$ cells, in the range reported in previous studies, can be obtained from nonmobilized peripheral blood. PET with [$^{18}$F]-fluorodeoxyglucose cell labeling is an efficient imaging method for homing assessment.** STEM CELLS *2006;24:333–336*

## INTRODUCTION

Adult myocardium has limited capacity to restore contractile function after infarction. Autologous hematopoietic stem cell (HSC) implantation in damaged myocardium has been proposed to prevent remodeling processes leading to cardiac insufficiency. HSC isolation from blood usually requires pretreatment with granulocyte–colony-stimulating factor (G-CSF). Administration of such factors in patients with acute myocardial infarction (AMI) is a matter of controversy. Several animal studies indicated a potentially beneficial role of G-CSF in the prevention of cardiac remodeling after AMI [1–4]. Human clinical studies, however, have led to the conclusion that G-CSF pretreatment is strongly associated with higher occurrence of instent restenosis and extension of the infarcted region [5, 6]. Some authors also reported that HSCs cannot differentiate into cardiomyocytes, leading to the concept that their myocardial restoration potential depends on other capacities [7–10]. In particular, CD34$^+$ cells could release growth factors activating cardiac stem cells; they also may differentiate into endothelial cells, leading to enhanced neoangiogenesis. Whatever the mechanism involved, it certainly requires CD34$^+$ cell homing in the injured tissue. Evaluations of cell trafficking and homing are therefore essential. They may be studied on a gamma camera after radiopharmaceutical cell labeling, but sensitivity and resolution would be much better with positron emission tomography (PET). Nevertheless, the short half-life of PET radiopharmaceuticals allows trafficking studies for only a few hours. We may therefore take advantage of the longer half-life of tracers

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used for gamma camera imaging to prolong cell detection for 2 to 3 days.

In this study, we tested the feasibility of peripheral-blood $CD34^+$ cell isolation without mobilization and applied PET and gamma camera imaging to assess their homing after intracoronary implantation in patients with AMI.

## Materials and Methods

### Patients

The selection criteria were a first acute anterior myocardial infarction with severe hypokinesis or akinesis of at least two adjacent segments on the baseline left ventricular echocardiogram and the absence of reperfusion within 6 hours after the onset of symptoms. After approval by the local ethics committee, six patients admitted in our institution gave their written informed consent and were included in the study. All patients were treated by aspirin, clopidogrel, statins, beta-blockers, and angiotensin-converting enzyme inhibitors. At the time of $CD34^+$ cell implantation, patients were 7 to 21 days after a documented AMI and 4 to 21 days after culprit coronary artery stenting. At 2 to 3 days before implantation, we assessed regional myocardial viability with PET after the injection of 370 MBq of [$^{18}$F]-fluorodeoxyglucose (FDG) in a hyperinsulinemic state. External marks were used to improve coregistration with the homing studies. Dobutamine stress echocardiography for assessment of viable myocardium was carried out before stem cell implantation. Regional left ventricular wall motion analysis was performed according to the American Society of Echocardiography recommendations [11], dividing the left ventricle into 17 segments to produce a wall motion score index.

### $CD34^+$ Cell Enrichment

Autologous peripheral blood HSCs were obtained after large-volume cytapheresis (15 to 20 L) performed on a Cobe Spectra cell separator. Positive selection of $CD34^+$ cells was performed on a Baxter Isolex 300i medical device. Selected cells were maintained at 4°C for approximately 20 hours and then labeled.

### Cell Labeling and Implantation

Two groups of 2 to 4 million $CD34^+$ cells were labeled with 74 MBq of FDG and 7.4 MBq of $^{111}$In-oxine, respectively, at the concentration of $2 \times 10^6$ cells/ml. Cells were subsequently washed in phosphate-buffered saline (PBS) and resuspended in 1 ml PBS containing 5% human albumin. During coronary angiography, labeled and unlabeled stem cells were injected through the central lumen of an over-the-wire balloon catheter (oversized by 0.5 mm) into the culprit coronary artery as described by Assmus et al. [12]. The balloon was inflated inside the stent with low pressure to completely block the blood flow during 3 minutes, and the $CD34^+$ cell suspension (2 ml) was infused distally to the occluding balloon through the central catheter port. After a washout with saline, the balloon was deflated for 3 minutes. The maneuver was repeated two to three times, and labeled stem cells were always used in the first infusion. This procedure allows for high-pressure infusions under stop-flow conditions to facilitate the infiltration of the cells into the infarcted zone and to prevent backflow. Enoxiparin 0.5 mg/kg was used during the implantation procedure.

### Homing Studies

One hour after implantation, 20-minute 3D PET images were obtained over the chest. For $^{111}$In imaging, 20-minute planar images were acquired on a gamma camera 19 hours and 43 hours after implantation. At 19 hours, tomographic acquisition was also performed.

### Follow-Up

Four months after implantation, patients were reevaluated by FDG-PET, echocardiography, and coronary angiography.

## Results

### $CD34^+$ Cell Enrichment

A mean of $32.4 \times 10^9$ (range, 22 to $40 \times 10^9$) mononuclear cells containing less than 0.1% $CD34^+$ cells were collected after treating a mean of 3.5 × total blood volume (range, 2.9 to 5.3). After $CD34^+$ selection, a mean of $14.9 \times 10^6$ (range, 3.6 to $27.6 \times 10^6$) cells containing a mean of 30% (range, 14%–35%) $CD34^+$ cells were obtained for implantation. Major contaminants were $CD19^+$ B-lymphocytes (mean, 42%; range, 29%–58%); monocytes were less than 1% of all cells.

### Implantation and Homing Studies

Mean (± standard deviation) labeling efficiency of implanted cells with $^{111}$In-oxine and FDG was 65% ± 8% and 6% ± 1%, respectively.

The stem cell implantation was well tolerated, without any complications, especially any early restenosis or arrhythmia (Table 1). One hour after implantation, 5.5% ± 2.3% of the administered label remained in the myocardium. The major part of the radioactive label was present in the liver (48% ± 35%), spleen (29% ± 19%), and bone marrow.

In four patients, detection of myocardial radioactivity was restricted to the vascular territory of the revascularized vessel, more precisely within the borders of the infarcted zone (Fig. 1). In one patient presenting with a large infarction and a no-reflow pattern, injected cells remained in a linear strip at the surface of the myocardium, suggesting a trapping in the left anterior descending artery. Apart from this observation, neither PET nor $^{111}$In imaging demonstrated cell homing in the patients' vasculature, including the aorta.

One patient exhibited cell homing in the middle part of the antero-septal region; this patient had small septal arteries emerging at the distal extremity of the stent. At 19 hours after implantation, $^{111}$In radioactivity was detected in the liver (71% ± 10%), spleen (29% ± 10%), and bone marrow. Only one of the six patients had detectable, although faint, activity in the myocardium. At 43 hours, the activity's distribution was similar.

### Patient Follow-Up

No restenosis or arrhythmia occurred in any patient. Echocardiography demonstrated enhancement in wall motion score index ($p < .05$) in all patients (Table 1), but left ventricular ejection fraction did not increase significantly (from 56% ± 7% to 60% ± 7%). FDG-PET follow-up studies demonstrated no alteration of the viable myocardium. A restricted and nonquantifiable extension of FDG uptake was detected in four patients.

**Table 1.** Clinical characteristics, $CD34^+$ cell preparation, PET imaging, and echocardiography findings in six patients with intracoronary $CD34^+$ cell implantation for acute myocardial infarction

| | | | Risk factors | | | | $CD34^+$ cells | | | | | PET | | Echocardiography WMSI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Apheresis | | | $CD34^+$ after selection | | Cardiac homing | | Before implantation | | Follow-up |
| Age | Sex | CCA | DM | HT | Hcho | Sm | Total blood (L) | MNC $\times 10^9$ | $CD34^+$ (%) | % | $\times 10^6$ | % | Localization | Baseline | Dobutamine | Baseline |
| 57 | M | 90% mid LAD | − | − | + | + | 15 | 35 | < 0.1 | 19 | 10.3 | 6 | infarction borders | 2.06 | 1.88 | 1.77 |
| 42 | F | 100% mid LAD | − | − | + | + | 15 | 35 | < 0.1 | 35 | 21 | 7 | small septal arteries territory | 1.77 | 1.77 | 1.18 |
| 65 | M | 100% mid LAD | − | − | + | + | 15 | 34 | < 0.1 | 21 | 9.5 | 4 | infarction borders | 1.71 | 1.59 | 1.53 |
| 71 | M | 100% proximal LAD | + | − | + | + | 17 | 22 | < 0.1 | 44 | 17.6 | 5 | LAD trapping | 1.65 | 1.53 | 1.53 |
| 59 | F | 100% mid LAD | − | + | + | − | 20 | 40 | < 0.1 | 46 | 27.6 | 7 | infarction borders | 1.41 | 1.41 | 1.41 |
| 54 | F | 100% proximal LAD | − | − | + | + | 20 | 28 | < 0.1 | 14 | 3.6 | 7 | infarction borders | 1.18 | 1.12 | 1.00 |

Abbreviations: CCA, culprit coronary artery; DM, diabetes mellitus; HT, hypertension. HCho, hypercholesterolemia; LAD, left anterior descending artery; MNC, mononuclear cells collected; PET, positron emission tomography; Sm, smoking; WMSI, wall motion score index at baseline and under dobutamine low dose (10 $\mu$g/kg per min), according to the American Society of Echocardiography recommendations (based on 17 left ventricular segments).

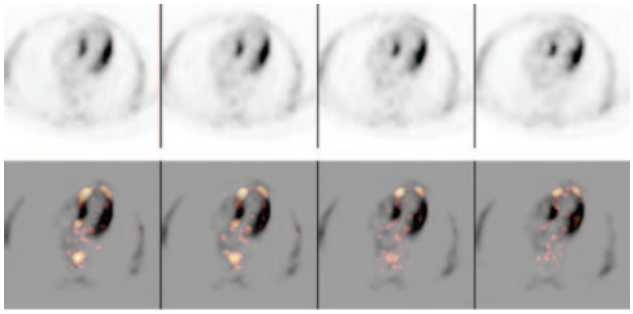

**Figure 1.** First row: successive transverse chest PET slices of FDG uptake testing the regional myocardial viability evaluation (gray scale). FDG uptake is markedly reduced in the antero-apical region of the left ventricle. Second row: superimposed on the PET-FDG slices presented in the first row, FDG-labeled $CD34^+$ cell homing is detected 1 hour after intracoronary injection in the hypometabolic infarcted area, except in the central core of the myocardial lesion (orange-to-yellow scale). Cells detected in the postero-median aspect of the chest correspond to $CD34^+$ cell homing in thoracic vertebral bone marrow. Abbreviations: FDG, fluorodeoxyglucose; PET, positron emission tomography.

## DISCUSSION

We have shown that large amounts of $CD34^+$ cells can be obtained from nonmobilized peripheral blood by large-volume cytapheresis followed by positive selection of $CD34^+$ cells. The amounts of $CD34^+$ cells available for therapeutic administration were in the range of those reported in previous studies [12, 13]. The procedure does not require mobilization by G-CSF and generates few monocytes (less than 1% of the selected cells). Although G-CSF permits the collection of much larger amounts of cells, this benefit is balanced by the risk of enhancing the restenosis process and the infarction extension, as reported in recent clinical studies [5, 6]. Our study therefore opens the way to clinical studies testing $CD34^+$ cell administration without the confounding effects of G-CSF. This study was not designed to assess the clinical efficacy of the cell-therapy protocol proposed. This would require a study on a larger number of patients and with a longer follow-up period. Such a study would also help confirm the inocuity of the procedure in terms of restenosis and arrhythmia occurrence.

Double radioactive labeling allowed for the assessment of cell trafficking and homing of injected cells. The sensitive and high-resolution PET study demonstrated deposition and early homing of $CD34^+$ cells in the myocardium surrounding the necrotic area. One patient exhibited cell homing in the middle part of the antero-septal region; as this patient had small septal arteries emerging just after the distal extremity of the stent, we postulate that labeled cell infusion preferentially occurred in these arteries rather than in the left anterior descending artery. As 4%–7% of the labeled cells were detected in the myocardium, we can reasonably conclude that $CD34^+$ cells constitute a major part of them because other labeled cells were essentially B-lymphocytes, a cell type that is not prone to adhere to, and migrate through, diseased coronary endothelium (monocytes were < 1% of all cells) [14, 15]. Cell trapping most probably occurred in the left anterior descending artery in one patient. Despite the report of bone marrow–derived progenitor cells homing in the aorta of atherosclerotic animals, we observed no other vascular labeling [16]. Apart from its component reflecting myocardial homing, the observed radioactivity distribution is in accordance with the normal homing of $CD34^+$ cells after intravascular injection. The imaging follow-up performed at 43 hours with the $^{111}$In gamma camera tracer suggested that the activity sequestrated outside the myocardium did not substan-

tially recirculate to secondarily reach the lesioned heart. Homing assessment is of major importance for comparison of cell-based therapeutic strategies. Satisfactory cell deposition and homing in the target area are indeed therapeutic prerequisites. They may greatly vary depending on the methods of cell collection, preparation, and implantation [17, 18]. The short half-life of the PET label that we used limited the duration of sensitive homing assessment. Still, the method revealed unexpected sites of deposition in two patients, indicating that individual factors also greatly influence cell distribution after intracoronary administration. This observation further strengthens the need for powerful cell imaging tools, such as PET, to correctly interpret cell-based trials that emerge in the various domains of regenerative medicine.

## CONCLUSION

Large amounts of $CD34^+$ cells, in the range reported in previous studies, can be obtained from nonmobilized peripheral blood. PET with FDG cell labeling is an efficient imaging method for homing assessment.

## ACKNOWLEDGMENTS

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## DISCLOSURES

The authors indicate no potential conflicts of interest.

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