Pilot Trial on Determinants of Progenitor Cell Recruitment to the Infarcted Human Myocardium

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Background—Clinical trials indicate a beneficial effect of intracoronary infusion of progenitor cells on myocardial function in patients with ischemic heart disease. The extent and potential determinants of proangiogenic progenitor cell homing into the damaged myocardium after intracoronary infusion and the underlying mechanisms are still unknown.

Method and Results—Circulating proangiogenic progenitor cells isolated from peripheral blood and cultivated for 3 days were labeled with radioactive indium oxine (111In-oxine). Radiolabeled proangiogenic progenitor cells (7.6±3.0 MBq, mean±SD) were administered to patients with previous myocardial infarction and a revascularized infarct vessel at various stages after infarction (5 days to 17 years). Viability of the infarcted myocardium was determined by 18F-fluorodeoxyglucose–positron emission tomography and microcirculatory function by intracoronary Doppler measurements. One hour after application of progenitor cells, a mean of 6.9±4.7% (range, 1% to 19%; n=17) of total radioactivity was detected in the heart, which declined to 2±1% after 3 to 4 days. Average activity within the first 24 hours was highest among patients with acute myocardial infarction (≈14 days; 6.3±2.9%; n=8) and progressively decreased in patients treated in an intermediate phase (>14 days to 1 year; 4.5±3.2%; n=4) or a chronic stage (infarct age >1 year; 2.5±1.6%; n=5). Low viability of the infarcted myocardium and reduced coronary flow reserve were significant (P<0.05) predictors of proangiogenic progenitor cell homing.

Conclusions—In patients after myocardial infarction undergoing intracoronary infusion of 111In-oxine–labeled proangiogenic progenitor cells, a substantial amount of radioactivity is detected for several days in the heart, indicating homing of progenitor cells to the myocardium. The amount of proangiogenic progenitor cells retained in the heart decreased progressively with time after the acute myocardial infarction. Proangiogenic progenitor cells preferentially home to extensive acute myocardial infarcts characterized by low viability and reduced coronary flow reserve. (Circulation. 2008;118:1425-1432.)

Key Words: heart failure ■ microcirculation ■ myocardial infarction ■ nuclear medicine ■ stem cells

Cell therapy is a promising option to treat ischemic diseases. Several cell types, including embryonic stem cells, skeletal myoblasts, bone marrow–derived cells, circulating progenitor cells (CPCs), and tissue-resident cardiac stem cells, have been used in experimental studies to improve recovery after acute ischemia and to repair chronically damaged myocardium.1 In the clinical setting, patients with acute and chronic myocardial infarction were treated with bone marrow–derived cells or blood-derived CPCs, whereas skeletal myoblasts were injected in chronic heart failure patients to improve heart function.1 The clinical trials in patients with acute myocardial infarction (AMI) and chronic ischemic heart disease were recently assessed in a meta-analysis of all studies currently available showing that cell therapy is associated with modest improvements in physiological and anatomic parameters above and beyond conventional therapy.2 In line with this, recent evaluation of the clinical follow-up of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial revealed a significant reduction in adverse clinical end points, setting the stage for future testing of clinical efficacy of cell therapy in AMI.3,4

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Despite these promising clinical studies, several questions about cell therapy remain unanswered, including the inten-
ively discussed question about the mechanism by which cell therapy exploits its beneficial effects. Direct physical contribution by differentiation into cardiac myocytes (cardiac regeneration) or the formation of new vessels (vasculogenesis) and paracrine activities of the transplanted cells have been considered to mediate the functional recovery of the heart after cell infusion.\(^5\) Of note, all of these mechanisms, even the short-term paracrine activities, depend on an initial homing and subsequent engraftment to the target tissue. Therefore, experimental and clinical studies attempt to investigate the initial homing by in vivo imaging. Noninvasive imaging has been performed by direct labeling with gamma-emitting radionuclides for gamma camera\(^a\) or single-photon emission computed tomography imaging.\(^7,8\) by using positron emitters such as \(^{[18]}\text{F}\)fluorodeoxyglucose (FDG) for positron emission tomography (PET),\(^9,10\) or by labeling of cells with iron particles for magnetic resonance imaging.\(^11-14\) All strategies were used successfully in experimental models with some limitations (for review, see Beeres et al\(^{15}\)). Clinically, labeling of bone marrow–derived cells or isolated CD133\(^+\) cells with the radiopharmaceutical \(^{111}\text{In-oxine was performed in several case reports in patients with AMI or chronic myocardial infarction.}^{16,17}\) Hofmann et al\(^{18}\) infused bone marrow–derived cells or CD34\(^+\) cells labeled with the PET tracer FDG into 3 patients with AMI. All experimental and clinical studies demonstrated a relatively low (ranging from 2% to 11%) acute accumulation of \(^{111}\text{In} in the heart in patients treated with bone marrow–derived cells or CD133\(^+\) cells. Only purified FDG-labeled CD34\(^+\) cells were shown to home more efficiently into the heart (14% to 39%).\(^{18}\) However, the reasons for the individual variation of cell homing are still unclear.

Here, we investigated the homing of intracoronary infused \(^{111}\text{In-oxine–labeled ex vivo cultured peripheral blood-derived CPCs in 17 patients with AMI and chronic myocardial infarction to determine potential predictors of homing. Cultivated circulating cells were chosen because of their lower sensitivity to irradiation-induced dysfunction and cell death.}^{6,19}\)

**Methods**

**Patients**

Twenty post–myocardial infarction patients were recruited between May 2005 and December 2006 into this single-center study. In 17 patients with an open infarct-related artery (either after stent implantation or bypass grafting), \(^{111}\text{In-oxine–labeled progenitor cells were infused, and subsequent imaging was performed. Infarct age ranged from 5 days to 17 years (8 patients with AMI within the last 14 days [≤14 days], 4 patients in an intermediate phase of >14 days to 1 year, and 5 patients with chronic ischemic heart disease [infarct age >1 year]). One patient with AMI was excluded because no gamma camera imaging could be performed. In 2 patients with an occluded infarct-related artery, labeled progenitor cells were infused into a noninfarcted vessel, providing angiographically visible collaterals to the occluded infarct vessel. In brief, exclusion criteria were defined as evidence for malignant disease, infectious or inflammatory disease, active bleeding, stroke within 2 years, surgery or trauma within 2 months, uncontrolled hypertension, renal or liver dysfunction, thrombocytopenia, or anemia. The ethics review board of the Hospital of the Johann Wolfgang Goethe University of Frankfurt, Germany, and the Federal Agency for Radiation Safety (Bundesamt für Strahlenschutz), Germany, approved the protocol, and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**CPC Preparation and Labeling**

Venous blood (250 mL) was collected, and mononuclear cells were purified and ex vivo cultured for 3 days as previously described by Assmus et al.\(^{20}\) Briefly, adherent cells were detached with 0.5 mmol/L EDTA, and \(~10^9\) CPCs were resuspended in a final volume of 5 mL growth factor and serum-free X-VIVO-10 medium (Biowhitetaker, Verviers, Belgium) to be labeled with \(~25\text{ MBq}^{111}\text{In-oxine (half-life, 2.8 days; γ energy, 171 keV, 245 keV, 37 MBq/mL, Tyco Healthcare, Neustadt, Germany})\) according to the manufacturer’s instructions with regard to pH adjustment and were incubated for 60 minutes at 37°C (see Aicher et al\(^{9}\)). Cells were washed twice with PBS. Then, labeled CPCs were resuspended in serum-free X-VIVO-10 medium and stored in a syringe in a total volume of 8 mL. Labeling efficacy was measured with a dose calibrator, and cell viability was assessed by trypan blue exclusion.\(^{19}\) The remaining unlabeled CPCs also were washed, resuspended in serum-free X-VIVO-10 medium, and stored in a separate syringe (with a total volume of 8 mL).

**Infusion Protocol**

CPCs were administered using the previously described stop flow technique.\(^{20,21}\) In brief, after arterial puncture, patients who were pretreated with aspirin and clopidogrel were given 7500 to 10 000 U heparin. Patients received both \(10^7\) radiolabeled CPCs and subsequently the remaining unlabeled CPC. First, \(4\text{ mL labeled CPC was infused via an over-the-wire balloon catheter advanced into the coronary artery supplying the infarct territory. Then, the balloon was inflated with low pressure to completely block blood flow for 3 minutes. After 3 minutes of reflow, the procedure was repeated. Subsequently, unlabeled CPCs were infused in 2 portions, 4 mL each, in the same way.**

**Doppler Measurements**

Before CPC administration, coronary blood flow velocity was assessed via an intracoronary Doppler wire (Flowire, Cardiometrics Inc, Mountain View, Calif) as previously described.\(^{22}\) In brief, the Doppler wire was advanced to the infarct artery and a noninfarcted, nonstenosed reference vessel (if available), and coronary flow was assessed at baseline and during maximal hyperemia induced by intracoronary adenosine infusion via the guiding catheter (2.4 mg/min).

Average peak velocity was recorded at baseline and during maximal hyperemia induced by adenosine, and coronary flow reserve (CFR) in each vessel was calculated as the ratio between adenosine-induced and basal average peak velocities. Relative CFR was calculated by dividing infarct vessel CFR by reference vessel CFR.

**FDG-PET Studies**

FDG-PET was performed with a whole-body PET scanner (ECAT EXACT 47, Siemens CTI, Malvern, Pa) as previously described.\(^{20}\) Standardized quantitative analysis of myocardial viability was performed with FDG-PET bull’s-eye views and by calculating the mean signal intensity in 17 myocardial segments. Segments supplied by the infarct area were defined according to the coronary anatomy.\(^{23}\) Finally, mean PET activity of all segments targeted by the artery supplying the infarct area was calculated.

**Monitoring of \(^{111}\text{In Activity******

Whole-body scintigraphy with a large-field double-head gamma camera equipped with high-resolution middle-energy collimators was used to detect radioactivity of infused \(^{111}\text{In-oxine–labeled CPC. Gamma camera studies using the same region of interest were...**
performed at 3 time points: 1 hour, 24 hours, and 3 or 4 days after intracoronary infusion of labeled CPCs. $^{111}$In activity was monitored for the whole body and the heart, liver, spleen, head, and bone marrow (region of interest within the os sacrum). $^{111}$In activity for these organs is corrected for decay and background activity and given as the percentage of total radioactivity. Average $^{111}$In activity within the first 24 hours was calculated as the area under the curve of $^{111}$In activity divided by the actual time difference between intracoronary infusion and the 24-hour measurement.

### Statistical Analysis

Continuous variables are presented as mean±SD (if not stated otherwise). In this descriptive pilot trial, no statistical analyses on subgroup comparisons were performed. Correlation of continuous data was performed by Spearman’s correlation coefficient. Statistical significance was assumed if values of $P<0.05$. All reported probability values are 2 sided. Statistical analyses were performed with SPSS (Version 14.0, SPSS Inc, Chicago, Ill).

### Results

Patient characteristics are summarized in the Table. A total number of $15±6.2×10^6$ progenitor cells, of which $≈10\%$ were labeled, were infused into the coronary artery of 17 patients with an over-the-wire balloon catheter. The average $^{111}$In activity of infused labeled CPCs was $7.6±3.0 \text{ MBq}$, and cell viability was $90±6.0\%$. $^{111}$In activity in the heart was highest 1 hour after application of CPCs ($6.9±4.7\%$; range, 1% to 19%) and progressively and substantially declined to $2±1\%$ after 3 to 4 days (Figures 1 and 2). Throughout the observation period of 3 to 4 days after intracoronary infusion of CPCs, a continuous level of $^{111}$In activity was detected in the liver and spleen, whereas $^{111}$In activity was highest immediately after infusion and progressively diminished in the lung and heart during the next days (Figure 2). Uptake in the bone marrow was low in all patients (Figure 2).

To determine whether infusion of CPCs into the collateral supplying artery is suitable to provide CPCs to infarcted areas with completely occluded infarct-related arteries, 2 additional patients received CPCs via the collateral supplying artery (see the Figure in the online-only Data Supplement). Average radioactivity within the first 24 hours in the heart was 3.0% in one of these patients and 9.0% in the other. However, gamma camera imaging suggested that significant $^{111}$In uptake was confined to the territory of the infused vessel, but no $^{111}$In uptake was observed in the target area supplied by the collaterals via retrograde filling of the occluded infarct-related artery.

### Predictors of CPC Homing

It is known that acute ischemia enhances the expression of chemoattractants and cytokines mediating homing of CPCs in experimental models. To determine whether acute ischemia indeed contributes to an augmented uptake of radioactively labeled cells, patients were stratified according to the interval.
between cell administration and time point of myocardial infarction. Figure 3A illustrates $^{111}$In activity in the heart of each individual patient along the time course separated into the 3 groups of infarct age. Average $^{111}$In activity in the heart within the first 24 hours was highest in patients receiving CPC infusion within 14 days ($\leq$14 days) after an AMI ($6.3 \pm 2.9 \%$), intermediate in patients receiving cells >14 days to 1 year after AMI ($4.5 \pm 3.2 \%$), and lowest in patients with healed infarcts that occurred >1 year before cell administration ($2.5 \pm 1.6 \%$). Thus, $^{111}$In uptake in the heart, expressed as an average of the measures within the first 24 hours, demonstrated a significant inverse correlation with the temporal interval between AMI and cell administration ($r = -0.59, P = 0.012; n = 17$) (Figure 3B), suggesting that the infarct age determines both uptake and retention of intracoronary infused radiolabeled cells. This association also was preserved when only patients with old infarcts (>1 year) were analyzed. In contrast, no association was found between $^{111}$In activity and infarct size as measured by the number of segments supplied by the infarct artery. Although homing of labeled cells was significantly higher in patients receiving progenitor cells within 1 year after AMI, Figure 3A illustrates the large variability of individual uptake of radioactivity in this patient population. Therefore, we
investigated the influence of viability of the infarcted myocardium as a measure of severity of myocardial damage on average $^{111}$In uptake in the heart. FDG-PET–determined viability of the infarcted myocardium was inversely correlated with a higher average $^{111}$In activity within the infarct territory (Figure 4). In contrast, in patients with healed myocardial infarction (>1 year old), no correlation of CPC homing was found as measured by average $^{111}$In activity and FDG-PET–derived myocardial viability.

When cells are applied via the coronary system, CPC adhesion and extravasation are required for homing and eventual tissue engraftment. To obtain insight into the potential role of the integrity of vascular function, coronary microcirculatory function was assessed after adenosine infusion in 11 patients with myocardial infarctions <1 year old before infusion of $^{111}$In-oxine–labeled cells. Average $^{111}$In activity after CPC infusion was highest in patients with impaired coronary microcirculatory function and declined with increasing CFR (Figure 5A). These results were confirmed if CFR was normalized to a noninfarcted reference vessel (Figure 5B).

Discussion

The results of the present descriptive pilot study demonstrate that 3 critical factors are likely to determine the uptake of labeled CPCs in the patient’s heart after intracoronary administration: infarct age, the extent of tissue damage, and the integrity of the coronary microcirculation. The data demonstrating a reduced homing of progenitor cells in chronic compared with AMI are well rationalized by the higher...
expression and release of chemoattracting cytokines during acute ischemia. AMI has experimentally been demonstrated to increase cytokines such as stromal cell-derived factor 1 (SDF-1), which enhances adhesion and transmigration of proangiogenic CPCs and hematopoietic stem cells. Recent studies additionally suggested that SDF-1 not only acts as a chemoattractant factor but also retains proangiogenic cells in the perivascular region. Likewise, SDF-1 serum levels are elevated in patients after AMI. In addition, hypoxia and inflammation as induced by myocardial infarction augment vascular endothelial growth factor and interleukin-8, both of which can attract subsets of progenitor cells. In human ischemic limb muscle, vascular endothelial growth factor, SDF-1, and CXCR4 expression was shown to be significantly augmented compared with nonischemic controls. Interestingly, when those investigators determined the expression of the cytokines in patients with chronic ischemia, the levels of vascular endothelial growth factor, SDF-1, and the SDF-1 receptor CXCR4 were significantly reduced below the levels of the nonischemic control muscles, indicating a severe reduction in these chemoattractants and cytokines in chronic ischemia. The finding that the extent of damaged tissue as assessed by FDG-PET measurement also correlated with increased CPC homing in AMI further supports the hypothesis that inflammatory processes and possibly necrotic tissue-derived factors act as chemoattractants in vivo. In addition, it is well established that progenitor cells derived from patients with chronic ischemic heart failure are functionally impaired. Indeed, although the small sample size of the present study precluded a more in-depth analysis, it is most likely that intracoronary infused progenitor cells derived from patients with chronic ischemic heart failure are less responsive to chemoattractants.

An impaired CFR measured directly before cell infusion was associated with an increased uptake of radiolabeled cells. An impairment in coronary vasodilator function after AMI is, among other reasons, associated with systemic inflammation and local leukocyte plugging, both indicating an inflamed and activated endothelium. Cytokine-mediated activation of the endothelium increases adhesion molecules, which act as binding partners to integrins expressed on the surface of progenitor cells. For example, β2 integrins are highly expressed on CPCs and bind to intercellular adhesion molecule-1, which is increased in dysfunctional endothelium. Although a cause-effect relationship obviously cannot be established by the present study, the importance of the ability of damaged myocardium to attract CPCs is underscored by a recent trial demonstrating that intracoronary infusion of bone marrow–derived progenitor cells normalizes impaired postinfarction CFR.

The biodistribution of radiolabeled cells and the extent of incorporation detected in the present study are consistent with previous case reports. With a similar labeling protocol, the 111In radioactivity in the heart was reported to be 2.6%, 4.9%, and 11% in 3 patients treated with bone marrow–derived mononuclear cells 4 to 7 days after AMI. Slightly lower incorporation (1.3% to 2.6%) was described in 3 patients who received bone marrow–derived cells labeled with FDG. It has been discussed whether a different mode of application may further enhance cell homing. Although to the best of our knowledge no information is available in the clinical setting, experimental studies demonstrated a slightly higher initial engraftment after intramuscular delivery of cells. In this study, the efficiency of interstitial retrograde coronary venous delivery was similar to that of coronary delivery. When we used collateral arteries to deliver cells to the nonviable infarcted tissue, migration through the collaterals appeared to be hampered. Thus, further studies should evaluate whether distinct delivery strategies are useful for improving the poor engraftment in humans. Alternatively (or additionally), augmentation of cell engraftment may be achieved by novel molecular strategies to increase homing and survival of the cells, particularly in chronic disease states. Experimental data provide promising results by preactivating the tissue by low-energy shock waves or by using biomaterials to release cytokines, thereby promoting engraftment and survival.

The present descriptive pilot trial using radioactive labeling is limited in sample size; therefore, no statistical comparisons of subgroups can be applied. A further limitation of the present study, shared with all clinically applicable noninvasive imaging techniques at present, is that we cannot ensure that the detected radioactivity equals living and functionally active cells. Although our preclinical studies demonstrated that free 111In in radioactivity is not taken up by infarcted hearts, we cannot exclude that 111In-labeled CPCs are phagocytosed by inflammatory cells. Moreover, radioactivity can...
be released by the labeled cells. However, this nonspecific release should most likely be similar in acute and chronic conditions and therefore may not be the cause of the significantly impaired cardiac uptake in chronic disease. Finally, the short-term monitoring of cells does not give insights into the long-term engraftment and function of the applied cells. However, without an initial homing, infused cells cannot exert either paracrine or regenerating activity. Therefore, the present study, with all its limitations, provides a basis for evaluating cell therapy—enhancing strategies in the future.

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Disclosures

Dr Schächinger has served as a consultant to t2cure. Drs Dimmeler and Zeiher have received research grants from DFG Cardiopulmonary Excellence Cluster and have ownership interest or have served as consultants to or on the advisory board of t2cure. The other authors report no conflicts.

References

This study investigates homing of radioactively labeled circulating progenitor cells into the infarct territory after intracoronary infusion into the infarct-related artery in patients after myocardial infarction. The salient findings are that the temporal proximity to the ischemic event, the extent of myocardial necrosis, and the magnitude of functional impairment of the coronary microcirculation are individually likely to determine an increased uptake and short-term retention of progenitor cells administered into the infarct-related coronary artery. These findings may indicate that the inflammatory processes ignited by the infarcted and necrotic myocardium may play a major role in the recruitment of progenitor cells into the ischemic tissue. Consequently, when the inflammatory activation of the coronary vasculature subsides during infarct healing and scar formation, recruitment and homing of intravascularly administered progenitor cells may be reduced.
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