Monitoring of Bone Marrow Cell Homing Into the Infarcted Human Myocardium

Michael Hofmann, MD, MS*; Kai C. Wollert, MD*; Gerd P. Meyer, MD; Alix Menke, MD; Lubomir Arseniev, MD; Bernd Hertenstein, MD; Arnold Ganser, MD; Wolfram H. Knapp, MD; Helmut Drexler, MD

Background—Intracoronary transfer of autologous bone marrow cells (BMCs) promotes recovery of left ventricular systolic function in patients with acute myocardial infarction. Although the mechanisms of this effect remain to be established, homing of BMCs into the infarcted myocardium is probably a critical early event.

Methods and Results—We determined BMC biodistribution after therapeutic application in patients with a first ST-segment–elevation myocardial infarction who had undergone stenting of the infarct-related artery. Unselected BMCs were radiolabeled with 100 MBq 2-[18F]-fluoro-2-deoxy-D-glucose (18F-FDG) and infused into the infarct-related coronary artery (intracoronary; n=3 patients) or injected via an antecubital vein (intravenous; n=3 patients). In 3 additional patients, CD34-positive (CD34+) cells were immunomagnetically enriched from unselected BMCs, labeled with 18F-FDG, and infused intracoronarily. Cell transfer was performed 5 to 10 days after stenting. More than 99% of the infused total radioactivity was cell bound. Nucleated cell viability, comparable in all preparations, ranged from 92% to 96%. Fifty to 75 minutes after cell transfer, all patients underwent 3D PET imaging. After intracoronary transfer, 1.3% to 2.6% of 18F-FDG–labeled unselected BMCs were detected in the infarcted myocardium; the remaining activity was found primarily in liver and spleen. After intravenous transfer, only background activity was detected in the infarcted myocardium. After intracoronary transfer of 18F-FDG–labeled CD34-enriched cells, 14% to 39% of the total activity was detected in the infarcted myocardium. Unselected BMCs engrafted in the infarct center and border zone; homing of CD34-enriched cells was more pronounced in the border zone.

Conclusions—18F-FDG labeling and 3D PET imaging can be used to monitor myocardial homing and biodistribution of BMCs after therapeutic application in patients. (Circulation. 2005;111:2198-2202.)

Key Words: bone marrow cells ■ imaging ■ myocardial infarction ■ nuclear medicine

It has been proposed that cardiac transfer of bone marrow cells (BMCs) can be used for cardiac tissue repair and regeneration in patients after acute myocardial infarction (AMI).1–3 This concept is supported by the recent randomized controlled BOne marrOw transfer to enhance ST-elevation infarct regeneratioN (BOOST) trial, showing that intracoronary transfer of unselected autologous BMCs during the early postinfarction period enhances recovery of left ventricular (LV) ejection fraction after 6 months.4 The mechanisms by which BMCs enhance functional recovery after AMI remain poorly understood. It has been proposed that bone marrow–derived endothelial progenitor cells may enhance neovascularization of ischemic tissues by differentiating to endothelial cells.5 In addition, recent articles have highlighted the potential of BMCs to secrete proangiogenic factors and suggested that paracrine signal-
Methods

Patient Population
Patients with a first ST-segment-elevation myocardial infarction who had undergone percutaneous coronary intervention with stent implantation of the infarct-related coronary artery and demonstrated hypokinesia or akinesia of more than two thirds of the LV anteroseptal, lateral, and/or inferior wall (as revealed by angiography immediately after percutaneous coronary intervention) were included in the study. Patients presenting with Killip class III and IV symptoms or multivessel coronary artery disease were excluded. The same inclusion and exclusion criteria were used in the BOOST trial.4 All patients provided written informed consent and received optimal postinfarction pharmacotherapy, including aspirin, clopidogrel, ACE inhibitors or angiotensin receptor blockers, β-blockers, and statins. The study was approved by our local ethics committee (No. 3297).

BMC Preparation
Five to 10 days after percutaneous coronary intervention, bone marrow was aspirated from the posterior iliac crest under a brief general anesthesia with etomidate and midazolam. Unselected BMCs were enriched under good manufacturing practice conditions by 4% gelatin-polysuccinate density gradient sedimentation as described.4 CD34

Results

Patient Characteristics
Nine patients (all male) were studied. Patient characteristics are summarized in Table 1. All patients tolerated BMC harvesting and transfer well. No bleeding complications at the harvest site were observed. There were no increases in troponin T serum levels in any of the patients 1 day after BMC transfer, indicating that the procedure did not inflict additional ischemic damage to the myocardium.

BMC Labeling With 18F-FDG
Labeling details are presented in Table 2. Nucleated cell viability, which was comparable in all preparations, ranged from 92% to 96%. In all applications, the activity in the supernatant after 3 steps of washing and centrifugation was 0.6±0.3% of the cell-bound activity.

Biodistribution of 18F-FDG–Labeled Unselected BMCs After Intracoronary Transfer (Protocol 1)
Radioactivity retention in the myocardium after intracoronary application of 18F-FDG–labeled unselected BMCs was 1.3% in patient 1, 2.6% in patient 2, and 2.3% in patient 3 (Table 2). In all 3 patients, scans covering the upper abdomen and chest were obtained. Full 3D regions of interest were generated with the MEDx 3.0 software (Medical Numerics). Myocardial activity was normalized to the sum of activity in liver, spleen, and heart.

Biodistribution of Unselected 18F-FDG–Labeled BMCs After Intravenous Injection (Protocol 2)
Intravenous application of 18F-FDG–labeled BMCs resulted in activity accumulation predominantly in liver and spleen (>84% of the activity in the field of view). Myocardium and adjacent mediastinal tissues were not resolved visually because of a lack of contrast (Table 2). When 18F-FDG–labeled
BMCs were subsequently infused intracoronarily in the same 3 patients, a significant retention in radioactivity was observed in the myocardium: 1.8% in patient 4, 4.3% in patient 5, and 5.3% in patient 6.

**Biodistribution of 18F-FDG–Labeled CD34-Enriched BMCs After Intracoronary Transfer (Protocol 3)**

Radioactivity retention in the myocardium after intracoronary application of 18F-FDG–labeled CD34-enriched BMCs was 39% in patient 7, 14% in patient 8, and 24% in patient 9 (Table 2). In all 3 patients, activity in the heart was localized in the area of the culprit vessel only. No significant activity was detected in other regions of the myocardium. More than 55% of the activity in the field of view accumulated in liver and spleen (example shown in the Figure, C and D, and Movie II). In contrast to unselected BMCs, which homed to the infarct center and border zone (Figure, A and B), homing of CD34-enriched BMCs was most prominent in the infarct border zone (Figure, C and D, and Movie II).

**Discussion**

Radiolabeling of leukocytes is an established nuclear medicine procedure to localize areas of infection and inflammation.

### TABLE 1. Demographic, Clinical, and Angiographic Characteristics of the Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>Risk Factors</th>
<th>Time From Symptom Onset to PCI, h</th>
<th>Infarct-Related Artery</th>
<th>Maximum CK Level, U/L</th>
<th>TIMI Flow Grade Before/After PCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>28.2</td>
<td>C, H</td>
<td>3</td>
<td>LAD</td>
<td>6480</td>
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<tr>
<td>2</td>
<td>39</td>
<td>26.1</td>
<td>F, H, S</td>
<td>11</td>
<td>LCx</td>
<td>1767</td>
<td>2/3</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>29.1</td>
<td>C, H</td>
<td>22</td>
<td>LAD</td>
<td>464*</td>
<td>2/3</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>25.6</td>
<td>C, S</td>
<td>3</td>
<td>RCA</td>
<td>1530</td>
<td>2/3</td>
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<tr>
<td>5</td>
<td>39</td>
<td>32.8</td>
<td>C, D, H</td>
<td>8</td>
<td>LAD</td>
<td>1228</td>
<td>0/3</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>27.2</td>
<td>F, S</td>
<td>27</td>
<td>LAD</td>
<td>905*</td>
<td>0/3</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>28.1</td>
<td>C, H</td>
<td>5</td>
<td>LAD</td>
<td>4804</td>
<td>0/3</td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>24.4</td>
<td>H</td>
<td>9</td>
<td>LAD</td>
<td>7479</td>
<td>2/2</td>
</tr>
<tr>
<td>9</td>
<td>41</td>
<td>28.7</td>
<td>S</td>
<td>3</td>
<td>LAD</td>
<td>5366</td>
<td>0/3</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; PCI, percutaneous coronary intervention; C, total cholesterol level >200 mg/dL; H, arterial hypertension; LAD, left anterior descending artery; F, family history of premature coronary artery disease; S, current smoking; LCx, left circumflex artery; D, diabetes mellitus; and RCA, right coronary artery.

*Because of late presentation of these 2 patients, CK levels measured on admission are shown.

### TABLE 2. BMC Preparation and 18F-FDG Labeling

<table>
<thead>
<tr>
<th>Patient</th>
<th>Initial BM Aspirate, mL</th>
<th>Cell Fraction</th>
<th>Volume, mL</th>
<th>NCs, ×10³</th>
<th>CD34⁺, ×10³</th>
<th>CD34⁺, %</th>
<th>Labeled Cell Population</th>
<th>Specific Activity, Bq/10⁶ NCs</th>
<th>Myocardial Homing,* % of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol 1: labeling of unselected BMCs followed by intracoronary transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>143</td>
<td>Unselected</td>
<td>29</td>
<td>36.7</td>
<td>14.9</td>
<td>0.55</td>
<td>5% of unselected BMCs</td>
<td>0.4</td>
<td>1.3</td>
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<tr>
<td>2</td>
<td>145</td>
<td>Unselected</td>
<td>26</td>
<td>21.1</td>
<td>13.5</td>
<td>0.57</td>
<td>5% of unselected BMCs</td>
<td>1.8</td>
<td>2.6</td>
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<tr>
<td>3</td>
<td>126</td>
<td>Unselected</td>
<td>25</td>
<td>18.5</td>
<td>8.7</td>
<td>0.62</td>
<td>5% of unselected BMCs</td>
<td>0.8</td>
<td>2.3</td>
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<tr>
<td>Protocol 2: labeling of unselected BMCs followed by intravenous (first half) and intracoronary (second half) transfer</td>
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<td></td>
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<tr>
<td>4</td>
<td>121</td>
<td>Unselected</td>
<td>25</td>
<td>20.9</td>
<td>10.4</td>
<td>0.50</td>
<td>5% of unselected BMCs</td>
<td>1.3</td>
<td>0 (after intravenous), 1.8 (after intracoronary)</td>
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<tr>
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<td>120</td>
<td>Unselected</td>
<td>28</td>
<td>25.5</td>
<td>4.0</td>
<td>0.14</td>
<td>5% of unselected BMCs</td>
<td>0.6</td>
<td>0 (after intravenous), 4.3 (after intracoronary)</td>
</tr>
<tr>
<td>6</td>
<td>131</td>
<td>Unselected</td>
<td>21</td>
<td>26.5</td>
<td>8.6</td>
<td>0.61</td>
<td>5% of unselected BMCs</td>
<td>1.0</td>
<td>0 (after intravenous), 5.3 (after intracoronary)</td>
</tr>
<tr>
<td>Protocol 3: labeling of CD34-selected cells followed by intracoronary transfer</td>
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<tr>
<td>7</td>
<td>336</td>
<td>CD34⁺</td>
<td>3</td>
<td>0.30</td>
<td>21.3</td>
<td>70.3</td>
<td>100% of CD34⁺ fraction</td>
<td>10</td>
<td>39</td>
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<tr>
<td></td>
<td>CD34⁻</td>
<td>90</td>
<td>45.5</td>
<td>6.4</td>
<td>0.14</td>
<td></td>
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<tr>
<td>8</td>
<td>320</td>
<td>CD34⁺</td>
<td>3</td>
<td>0.20</td>
<td>14.7</td>
<td>74.4</td>
<td>100% of CD34⁺ fraction</td>
<td>18</td>
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<td>CD34⁻</td>
<td>110</td>
<td>42.5</td>
<td>1.3</td>
<td>0.03</td>
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<td>9</td>
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<td>CD34⁺</td>
<td>3</td>
<td>0.22</td>
<td>12.0</td>
<td>55.0</td>
<td>100% of CD34⁺ fraction</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>CD34⁻</td>
<td>60</td>
<td>41.8</td>
<td>5.2</td>
<td>0.13</td>
<td></td>
<td></td>
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</table>

BM denotes bone marrow; NCs, nucleated cells.

*P<0.0036 for interprotocol comparisons (nonparametric exact Kruskal-Wallis test).
Myocardial homing and biodistribution of $^{18}$F-FDG–labeled BMCs. Left posterior oblique (A) and left anterior oblique (B) views of chest and upper abdomen of patient 2 taken 65 minutes after transfer of $^{18}$F-FDG–labeled, unselected BMCs into left circumflex coronary artery. BMC homing is detectable in the lateral wall of the heart (infarct center and border zone), liver, and spleen. Left posterior oblique (C) and left anterior oblique (D) views of chest and upper abdomen of patient 7 taken 70 minutes after transfer of $^{18}$F-FDG–labeled, CD34-enriched BMCs into left anterior descending coronary artery. Homing of CD34-enriched cells is detectable in the anteroseptal wall of the heart, liver, and spleen. CD34$^+$ cell homing is most prominent in infarct border zone (arrowheads) but not infarct center (asterisk).

in patients. Of the different labeling strategies for identification of inflammatory foci, $^{18}$F-FDG PET provides superior image quality with high spatial resolution and adequate scatter and attenuation correction. As shown in the present study, $^{18}$F-FDG labeling followed by 3D PET imaging can be used to monitor myocardial homing and biodistribution of BMCs after therapeutic application in post-AMI patients.

Intracoronary delivery of unselected BMCs has recently been shown to enhance LV ejection fraction recovery in patients after AMI. Using the same methodology for isolation and intracoronary transfer of unselected BMCs that was used in the BOOST trial, we have shown that only a small fraction (1.3% to 2.6%) of the transplanted cells are actually retained in the infarcted myocardium, whereas most cells home to the liver and spleen within 1 hour after intracoronary delivery. Selective, stop-flow balloon catheter delivery of BMCs resulted in BMC homing only in myocardial regions perfused by the infarct-related artery. Assuming that it is the engrafted cell population that promotes functional recovery after AMI, we postulated that $^{18}$F-FDG labeling can be used to explore alternative, less invasive routes of BMC application after AMI. However, after intravenous application of $^{18}$F-FDG–labeled BMCs, no activity retention was detected in the myocardium. This finding may not be too surprising when we consider that no coronary stop-flow technique was used and that only $\approx 4\%$ to 5% of cardiac output passes through the coronary arteries (even less through the culprit vessel). Similar to the situation after intracoronary BMC transfer, liver and spleen were the major sites of BMC homing after intravenous application, which is reminiscent of leukocyte scanning in which liver and spleen very effectively extract white blood cells during first passage. These data indicate that intracoronary transfer may be the preferred route for therapeutic BMC delivery in patients after AMI, although the limited time interval between administration and imaging did not allow us to exclude late redistribution of cells to the infarcted myocardium.

Unselected BMCs represent a mixed population of various stem and progenitor cells, stromal cells, and hematopoietic cells at various maturation stages. It is not yet clear which cell population(s) promote functional recovery in patients after intracoronary transfer. In experimental models of AMI, functional improvements have been reported after transplantation of unselected or highly selected BMC populations, including endothelial progenitor cells, hematopoietic stem cells, and mesenchymal stem cells. Importantly, by delivering proangiogenic factors, subsets of mature hematopoietic cells may cooperate with transplanted or resident cardiac stem and progenitor cells to enhance their capacity for tissue repair after ischemic injury.

After myocardial ischemia and reperfusion, neutrophils and monocytes are recruited to the infarcted myocardium as part of a cell-mediated inflammatory response, suggesting that myocardial homing of $^{18}$F-FDG–labeled unselected BMCs may reflect, to some degree, homing of such differentiated cell types. However, as our study shows, cells expressing the stem and progenitor cell surface marker CD34 are selectively engrafted in the infarcted human myocardium. In fact, CD34-enriched cells displayed a higher retention in the infarcted myocardium compared with unselected BMCs. However, this may be due in part to the fact that, on average, 5-fold more $^{18}$F-FDG–labeled compared with CD34-enriched BMCs were infused intracoronarily, which may have led to a saturation of binding sites by unselected BMCs. Interestingly, CD34-enriched cells predominantly homed in the infarct border zone. Notably, intracoronary BMC transfer enhances LV contractility primarily in myocardial segments adjacent to the infarcted area, consistent with the idea that homing of CD34$^+$ BMCs corresponds to late functional improvement.

Compared with other PET tracers, $^{18}$F-FDG has a number of important advantages, including availability and ease of use, very low concentrations needed for PET imaging (femtomolar range), and long positron range of the emitted $\beta$-particle ($\approx 0.5$ mm), which results in a radiation dose deposition mostly outside the labeled cells. Moreover, the $511$-keV $\gamma$-photons emitted after positron annihilation display very low absorption in water and contribute $<5\%$ to the dose absorbed. Because the exposure of cells to radioactivity is highest during the labeling step and very low after transfer into the patient, the major dosage is absorbed during labeling and purification. Using the MIRD 3.1 software package, we estimated that the dose distributed in the labeling volume is $<0.05$ Gy. At a hematocrit of 10% (for BMCs), the homogeneously suspended cells absorb only a fraction of the energy deposited, leading to radiation doses of $<0.005$ Gy. This dose is well within the range of other nuclear medicine applications such as $^{18}$F-FDG PET or $^{11}$In-octreotide.
scintigraphy. This is in contrast to the frequently used auger-electron emitter tracer $^{111}$In-oxine, which, because of the short range of the emitted low-energy $\beta^-$-particles, delivers most of its radiation dose inside the labeled cells. This may explain why higher cellular toxicity has been observed with this tracer.

Because of the physical half-life (110 minutes) of $^{18}$F, we could not assess cell trafficking and myocardial retention of BMCs days after therapeutic application. Other PET isotopes with longer half-lives like $^{125}$I, $^{185}$Y, $^{64}$Ga, or $^{64}$Cu have unfavorable branching ratios or additional image-disturbing emissions but may be worth pursuing because of the small doses needed to monitor cell distribution. Another way to gain insight into more delayed time frames is to use PET scanners with higher performances at very low intracorporal activity concentrations that display improved intrinsic signal-to-noise ratios (e.g., LSO or GSO detectors). With such techniques, time points up to 10 hours after cell application (5 times the physical half-life of $^{18}$F) would be accessible for quantification.

Whether the amount of BMCs or CD34$^+$ cells retained in the infarcted myocardium is correlated to later improvement in LV contractile function will have to be addressed in future studies. If that were the case, $^{18}$F-FDG PET scanning could be used to screen various pharmacological or genetic approaches that prepared the bone marrow cells. Dr Arseniev has not been involved in data analyses. Whether the amount of BMCs or CD34$^+$ cells retained in the infarcted myocardium is correlated to later improvement in LV contractile function will have to be addressed in future studies. If that were the case, $^{18}$F-FDG PET scanning could be used to screen various pharmacological or genetic approaches that prepared the bone marrow cells. Dr Arseniev has not been involved in data analyses.

Acknowledgment

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Disclosure

Dr Arseniev is business leader of Cytonet Hannover, the company that prepared the bone marrow cells. Dr Arseniev has not been involved in data analyses.

References


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