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Bone Marrow–Derived Mesenchymal Cell Mobilization by Granulocyte-Colony Stimulating Factor After Acute Myocardial Infarction

Results From the Stem Cells in Myocardial Infarction (STEMMI) Trial

Rasmus Sejersten Ripa, MD; Mandana Haack-Sørensen, MSc; Yongzhong Wang, MD, PhD; Erik Jørgensen, MD; Steen Mortensen, Tech; Lene Bindslev, MSc, PhD; Tina Friis, MSc, PhD; Jens Kastrup, MD, DMSc

Background—Granulocyte-colony stimulating factor (G-CSF) after myocardial infarction does not affect systolic function when compared with placebo. In contrast, intracoronary infusion of bone marrow cells appears to improve ejection fraction. We aimed to evaluate the G-CSF mobilization of subsets of stem cells.

Methods and Results—We included 78 patients (62 men; 56±8 years) with ST-elevation myocardial infarction treated with primary percutaneous intervention <12 hours after symptom onset. Patients were randomized to double-blind G-CSF (10 µg/kg/d) or placebo. Over 7 days, the myocardium was exposed to 25×10⁶ G-CSF mobilized CD34⁺ cells, compared with 3×10⁶ cells in placebo patients (P<0.001); and to 4.9×10¹¹ mesenchymal stem cells, compared with 2.0×10¹¹ in the placebo group (P<0.001). The fraction of CD34⁺ cells/leukocyte increased during G-CSF treatment (from 0.3±0.2 to 1.1±0.9×10⁻³, P<0.001 when compared with placebo), whereas the fraction of putative mesenchymal stem cells/leukocyte decreased (from 22±17 to 14±11×10⁻³, P=0.01 when compared with placebo). An inverse association between number of circulating mesenchymal stem cells and change in ejection fraction was found (regression coefficient −6.8, P=0.004), however none of the mesenchymal cell subtypes analyzed, were independent predictors of systolic recovery.

Conclusions—The dissociated pattern for circulating CD34⁺ and mesenchymal stem cells could be attributable to reduced mesenchymal stem cell mobilization from the bone marrow by G-CSF, or increased homing of mesenchymal stem cells to the infarcted myocardium. The inverse association between circulating mesenchymal stem cells and systolic recovery may be of clinical importance and should be explored further. (Circulation. 2007;116[suppl I]:I-24–I-30.)

Key Words: stem cells • angiogenesis • heart failure • magnetic resonance imaging • myocardial infarction

Granulocyte-colony stimulating factor (G-CSF) therapy, with the mobilization of bone marrow stem cells soon after ST-elevation acute myocardial infarction in the Stem Cells in Myocardial Infarction (STEMMI) trial, did not improve left ventricular systolic function when compared with placebo.1,2 These results have been confirmed by the REVIVAL-2 trial3 and the G-CSF-STEMI trial.4 G-CSF was hypothesized to have beneficial effects on the myocardium both indirectly through the mobilization of bone-marrow stem cells into the peripheral circulation, and also perhaps directly by inhibiting myocardial apoptosis.5 Direct infusion of bone marrow mononuclear cells into the coronary arteries after an acute myocardial infarction has been investigated in several medium sized clinical trials,6–8 but only 2,9,10 are randomized, double-blind, and placebo-controlled. The REPAIR-AMI trial suggested a significant improvement of left ventricular ejection fraction,9 whereas only regional systolic function appeared to improve after intracoronary infusion of bone marrow mononuclear cells in the trial by Janssens et al.10 The apparently contradictory results of direct intracoronary infusion of bone marrow–aspirated cells versus pharmacological mobilization of bone marrow–derived stem cells are puzzling. G-CSF is known to mobilize cells from the hematopoietic cell line,11 and recent evidence suggests that bone marrow–derived mesenchymal stem cells, in particular, have cardiac reparative properties.12

The primary end point of the published STEMMI trial was change in regional systolic wall thickening from day 1 to 6 months as evaluated by cardiac magnetic resonance imaging (MRI). Changes in ejection fraction by MRI were a secondary end point.1

The objective of this substudy was to describe (1) the cells mobilized by G-CSF with special emphasis on mesenchymal...
stem cells, and (2) the association between the plasma concentration of the cells and subsequent changes in left ventricular systolic function.

Methods

Study Design
All 78 patients from the STEMMI trial were included for analyses. Details of the study design and inclusion criteria have been published previously. Briefly, patients were included if they had a first time ST-elevation myocardial infarction (STEMI) successfully treated with primary percutaneous coronary intervention within 12 hours after the onset of symptoms. Patients were randomized to double-blind treatment with subcutaneous G-CSF (Neupogen, Amgen Europe BV, Breda, The Netherlands; 10 μg/kg body weight) or a similar volume of placebo (isotonic sodium-chloride) once daily for 6 days. The study was approved by the local ethical committee (KF 01 to 239/02), the Danish Medicines Agency (2612–2225), and was registered in clinicaltrials.gov (NCT00135928). All patients received verbal and written information about the study, and gave theirsigned consent before inclusion.

Quantification and Characterization of Stem Cells
The concentration of circulating CD34-positive (CD34+) cells and circulating putative mesenchymal stem cells in the blood quantified as CD45 and CD34 double-negative (CD45-CD34+) cells was measured by multiparametric flow cytometry using anti-CD45 (Becton Dickinson) and anti-CD34 (BD), as previously described. A panel of monoclonal and polyclonal antibodies was used to further characterize mesenchymal stem cells, including anti-CD105 (Endoglin; Ancell), anti-CD31 (platelet endothelial cell adhesion molecule; BD), anti-CD133 (hematopoietic stem cell antigen; Miltenyi Biotec), anti-CD73 (BD), anti-CD166 (activated leukocyte adhesion molecule; BD), anti-CXC chemokine receptor 4; R&D Systems), anti-VEGF R2 (R&D Systems), and anti-CD144 (VE-cadherin; BenderMed). Analytic gates were used to enumerate the total number and subsets of circulating CD45-CD34+ cells. Cell suspensions were evaluated by a FACS Calibur (BD). At least 100,000 cells per sample were acquired. Analyses were considered informative when adequate numbers of CD45-CD34+ events (300–400) were collected in the analytic gate.

The mean number of both CD34+ cells and CD45-CD34+ cells supplied to the postischemic myocardium by the blood during the first week after the treatment was approximated. First, we calculated the mean plasma concentration of cells during the first week: [(concentration day 1)+(concentration day 4)+(concentration day 7)]/3 (assuming a near linear increase in concentration). Next, we estimated the blood flow through the infarct related artery during one week (4800 mL/hr×24 hour/7 days). Finally, to get a rough estimate of the number of cells passing through the infarct related artery during 1 week, we multiplied the blood flow with the “mean plasma cell concentration” in each patient.

TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=39)</th>
<th>G-CSF (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54.7±8.1</td>
<td>57.4±8.6</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>34 (87)</td>
<td>28 (72)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.1±3.9</td>
<td>27.4±4.4</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>4 (10)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Known hypertension, n (%)</td>
<td>10 (26)</td>
<td>13 (33)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>31 (80)</td>
<td>22 (56)</td>
</tr>
<tr>
<td>Median maximum serum CK-MB concentration (quartiles), μg/L</td>
<td>274 (141–441)</td>
<td>320 (194–412)</td>
</tr>
<tr>
<td>Median time from symptom debut to primary percutaneous coronary intervention. (quartiles), h</td>
<td>4.23 (3.32–6.44)</td>
<td>3.47 (2.43–5.25)</td>
</tr>
</tbody>
</table>

Plasma Cytokines
Plasma concentrations of vascular endothelial growth factor A (VEGF-A) and stromal cell-derived factor 1 (SDF-1) concentrations were measured in duplicate by a colorimetric ELISA kit (R&D Systems). The lower limits of detection were 10 pg/mL for VEGF-A and 18 pg/mL for SDF-1, respectively.

Cardiac MRI
MRI was performed at baseline and 6 months after inclusion as previously described in details. In short, cine and late contrast-enhancement images were obtained with a 1.5-T scanner (Siemens Vision Magnetom, Siemens AG). The examinations were analyzed by an independent core laboratory (Bio-Imaging Technologies B.V.) using the MRI-MASS v6.1 (MEDIS Medical Imaging Systems). The core laboratory was blinded to all patient-data. MRI was feasible in 28 and 31 patients in the placebo and the G-CSF group, respectively.

Statistical Analyses
Data were analyzed using SPSS 13.0. The numbers of cells in the 2 treatment groups were compared using Mann-Whitney U test. The effects of the G-CSF treatment on cell, SDF-1, and VEGF-A concentrations were analyzed in a 2-factor ANOVA with repeated measures as a within-subject factor and treatment group as a between-subjects factor after logarithmic transformation to assume normal distribution if appropriate. Associations between number of cell supplied to the postischemic myocardium and change in left ventricular systolic function were determined using linear regression models with type of treatment included as covariate in all analyses. All data are expressed as mean±SD unless otherwise stated. All tests were 2-sided, and statistical level of significance was set at P<0.05. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Patients
Main baseline characteristics are shown in Table 1, a detailed description has been published previously. We included 39 patients in each group. In the placebo group, 3 patients withdrew consent and 1 patient died, and 1 patient in the G-CSF group withdrew consent before completion of the study treatment. Thus, cell data pertaining to the placebo and G-CSF were available from 35 and 38 patients, respectively.

Mobilized Cells
The G-CSF treatment led to a substantial increase in the plasma concentration of leukocytes (from 8.3±2.2 to 51.2±18.0×10⁹/mL) and CD34+ cells (supplemental Table I, available online at http://circ.ahajournals.org), with a peak
value 7 days after initiation of G-CSF therapy and normalization after 30 days. Thus, over 7 days, the postischemic myocardium was exposed to approximately $25\pm20\times10^9$ circulating CD34$^+$ cells, compared with approximately $3.2\pm1.4\times10^9$ cells in the patients receiving placebo ($P<0.001$).

The number of circulating putative mesenchymal stem cells (CD45$^-$/CD34$^+$) increased approximately 4-fold (supplemental Table I) during G-CSF treatment, resulting in myocardial exposure to $5.0\pm3.7\times10^9$ cells after G-CSF treatment compared with $2.0\pm1.2\times10^9$ in the placebo group ($P<0.001$).

Figure 1 shows the number of CD34$^+$ cells (panel A) and CD45$^-$/CD34$^+$ (panel B) relative to the leukocytes. The fraction of CD34$^+$ cells increased during G-CSF treatment, whereas the fraction of CD45$^-$/CD34$^+$ cells surprisingly decreased during the treatment. The fraction of mononuclear cells in the blood without the CD45 marker but with the CD34 marker (CD45$^-$/CD34$^+$) was unaffected by the G-CSF treatment ($P=0.1: 0.14\pm0.09$ versus $0.19\pm0.14$ per 1000 leukocytes at day 7 in the placebo and G-CSF groups, respectively).

The CD45$^-$/CD34$^+$ mesenchymal stem cells were subcharacterized by early stem cell markers (CD105, CD73, CD166, CXCR4), endothelial markers (CD31, CD144, VEGFR2), or a hematopoietic marker (CD133). Figure 2 shows the relative change from baseline to day 7 in both the placebo and the G-CSF groups. Most of the mesenchymal stem cells subfractions were increased (had a value above 1) in the placebo group and reflect the natural course after a myocardial infarction. However, the fraction of CD45$^-$/CD34$^+$ cells with the early stem cell marker CD73 had a significantly lower increase in the G-CSF group compared with the increase in the placebo group; and the fraction with endothelial marker CD31 was significantly decreased in the G-CSF treated group. As expected, cells with the hematopoietic marker CD133 increased significantly more in the G-CSF group than in the placebo group. Furthermore, it is apparent from Figure 3 that most of the sub-cell fractions tended to decrease during G-CSF treatment.

**Change in Left Ventricular Systolic Function and Stem Cell Mobilization**

There was no association between the total number of CD34$^+$ cells supplied to the postischemic myocardium after myocardial infarction and the subsequent change in left ventricular ejection fraction (Figure 4A; 95% CI of regression coefficient $-8.5$ to $1.5$, $P=0.2$).

An inverse association was found between the number of CD45$^-$/CD34$^+$ cells supplied to the postischemic myocardium and the change in left ventricular ejection fraction (Figure 4B) (95% CI of regression coefficient $-11.4$ to $-2.2$, $P=0.001$).
This association remained significant when controlling for infarct size, plasma concentration of SDF-1, plasma concentration of VEGF, and leukocyte concentration. The association was not reproduced when using systolic wall thickening in the infarct area (regression coefficient $-0.08$, $P=0.2$), the infarct border area (regression coefficient $-0.08$, $P=0.1$), or infarct size (regression coefficient $2.04$, $P=0.3$) as outcome. There was no significant interaction between type of treatment and number of CD45$^+$/CD34$^+$ cells indicating that the association was not significantly affected by the G-CSF treatment.

Next, we examined the association between the subtypes of CD45$^+$/CD34$^+$ cells and changes in left ventricular ejection fraction (Table 2). None of the subtypes were independent predictors when controlling for treatment type and the total number of CD45$^+$/CD34$^+$ cells. Only cells with the hematopoietic marker CD133 tended to have a positive association with the systolic improvement ($P=0.07$).

### Change in SDF-1 and VEGF-A Plasma Concentrations

The individual time course of homing factor SDF-1 and the vascular growth factor VEGF-A plasma concentrations are shown in supplemental Figure I. As previously shown, the SDF-1 time course differed significantly between the G-CSF and placebo groups ($P<0.001$), whereas the VEGF-A time course were similar ($P=0.4$). Classic cardiovascular risk factors (diabetes mellitus, smoking, age, and gender) as well as time to reperfusion and infarct size did not relate to the plasma concentration of SDF-1 by linear regression analysis, but high VEGF concentration at day 4 and 7 were significant.

<table>
<thead>
<tr>
<th>Cells in 10^9 per ml</th>
<th>Regression Coefficient*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45$^+$/CD34$^+$</td>
<td>$-0.15$</td>
<td>$-0.38$ to $0.38$</td>
</tr>
<tr>
<td>CD45$^+$/CD34$^+$/CD105$^+$</td>
<td>$-1.13$</td>
<td>$-3.45$ to $1.18$</td>
</tr>
<tr>
<td>CD45$^+$/CD34$^+$/CD166$^+$</td>
<td>$0.50$</td>
<td>$-0.30$ to $1.29$</td>
</tr>
<tr>
<td>CD45$^+$/CD34$^+$/CD31$^+$</td>
<td>$-0.09$</td>
<td>$-0.25$ to $0.8$</td>
</tr>
<tr>
<td>CD45$^+$/CD34$^+$/CXCR4$^+$</td>
<td>$0.01$</td>
<td>$-0.04$ to $0.07$</td>
</tr>
<tr>
<td>CD45$^+$/CD34$^+$/CD114$^+$</td>
<td>$-0.03$</td>
<td>$-0.20$ to $0.14$</td>
</tr>
<tr>
<td>CD45$^+$/CD34$^+$/VEGF-R$^+$</td>
<td>$0.21$</td>
<td>$-0.22$ to $0.63$</td>
</tr>
<tr>
<td>CD45$^+$/CD34$^+$/CD133$^+$</td>
<td>$1.23$</td>
<td>$-0.10$ to $2.57$</td>
</tr>
</tbody>
</table>

*Type of treatment and total number of CD45$^+$/CD34$^+$ are included as covariates in all models.
cantly related to long time to reperfusion (regression coefficient 43.2, \( P=0.002 \)).

There were no apparent association between either CD45/CD34 or CD34+ and VEGF-A (supplemental Table II), whereas both cell types were negatively associated with SDF-1 on all 3 days in the G-CSF group. Only CD34+ seemed to be negatively associated with SDF-1 in the placebo group, whereas CD45-CD34+ was not.

Neither SDF-1 nor VEGF-A concentrations in the week after the STEMI predicted the recovery of ejection fraction (Figure 5). In addition, there was no demonstrable association between leukocyte concentration and recovery of ejection fraction (\( P=0.4 \)).

**Discussion**

This trial demonstrated that G-CSF treatment induced a dissociated pattern in circulating CD34+ mononuclear cells and CD45+/CD34+ mononuclear cells (mesenchymal) with higher concentration per leukocyte of CD34+ compared with CD45-/CD34- cells. In addition, treatment with G-CSF causes a shift in the subtypes of mesenchymal stem cells in the peripheral blood. Finally, the numbers of circulating mesenchymal stem cells appeared to predict the change in left ventricular ejection fraction after STEMI.

The initial optimism regarding the application of stem cell therapy to ischemic heart disease after the preclinical and early clinical studies has been dampened recently by larger clinical trials designed to investigate efficacy. Intracoronary infusion of ex vivo purified bone marrow mononuclear cells might have effects on left ventricular recovery after STEMI, even though results are still scattered.\(^9,10\) G-CSF therapy to mobilize bone marrow stem cells, however, has failed to improve left ventricular restoration in 3 double-blind placebo-controlled trials using several end points.\(^1,3,4\)

It seems paradoxical that in vivo mobilization of bone marrow-derived cells to the circulation does not result in myocardial recovery comparable to that achieved by ex vivo purification and subsequent intracoronary infusion of bone marrow-derived cells. There may be several reasons for this. For example, G-CSF may not mobilize effective cell types. This hypothesis would be difficult to test in humans, however, because (1) the cells infused intracoronary are very heterogeneous\(^15\) and the cell(s) with potential for cardiac repair have not been established; and (2) G-CSF mobilization is difficult to assess because the measured concentration of circulating stem cells is dependent on both the mobilization and the homing of cells to the infarcted myocardium. Recent animal experiments indicate that mesenchymal stem cells may be good candidates for cardiac repair,\(^12,16\) whereas the hematopoietic cells (CD34+) are less likely to improve cardiac function.\(^17\) The information presented here supports a dissociated G-CSF mobilization of CD34+ cells compared with mesenchymal stem cells which may indicate a different therapeutic potential of G-CSF stem cell mobilization versus intracoronary purified cell infusions.

Furthermore, if cells are not directly responsible for the treatment effect after intracoronary infusion of bone marrow solution, but rather it is substances such as paracrine factors secreted by the cells,\(^18,19\) this might also explain some of the differences when compared with G-CSF treatment. Also, recent evidence suggests that G-CSF treatment impairs the migratory response to SDF-1 of endothelial progenitor cells.\(^20\) It is thus of importance to include measures of functional capacities in future cell trials.

The hypothesis of the STEMMI trial was that G-CSF mobilized CD34+ or CD45+/CD34- would home to and engraft into infarcted myocardium and participate in neovascularization and perhaps cardiomyocyte regeneration. This study demonstrated that there was no statistical significant association with the calculated total number of CD34+ cells, and an inverse association with the calculated CD45-/CD34- cells delivered to the myocardial perfusion during G-CSF treatment and the subsequent improvement in left ventricular ejection fraction. These results may indicate that a low concentration of the mesenchymal stem cells in the blood is attributable to engraftment of the cells into the myocardium. Thus, patients with a high inert potential for myocardial homing of the mesenchymal stem cells after STEMI will have the highest degree of systolic recovery attributable to the engrafted stem cells. We measured the plasma concentration of cytokines SDF-1 and VEGF as indicators of inert homing potential. Neither of these cytokines appeared to influence the recovery of ejection fraction, but a high concentration of SDF-1 was associated with a low concentration of cells indicating that SDF-1 may increase homing of the cells. However, plasma concentrations of the cytokines are proba-
bly poor indicators of the concentrations within the myocardium, but a more precise measurement would require repeated catheterizations of the patients. Recent evidence suggest that acute myocardial ischemia does not upregulate SDF-1 gene expression in human heart shortly after ischemia and reperfusion, whereas VEGF-A gene expression seemed upregulated after reperfusion. It would be very interesting to label mesenchymal stem cells within the bone marrow, and then follow their potential engraftment within the myocardium during G-CSF treatment.

If mobilization of CD34+ cells does not contribute to myocardial recovery after STEMI, and if circulating CD45 / CD34+ cells are not homing but potentially reduce the recovery of the myocardial function, this may also explain the inverse association between circulating mesenchymal stem cells and changes in ventricular function. However, the result could also be a random finding, because we could not demonstrate any correlation to changes in regional systolic function, and the calculated total amount of cells during G-CSF treatment is an estimate of cardiac exposure to stem cells based on several assumptions.

Previously, a study in dogs has indicated that intracoronary infusion of mesenchymal stem cells could cause microinfarctions, probably attributable to microvascular obstruction by the cells. However, circulating mononuclear cells collected after G-CSF treatment and then injected into the infarct related coronary artery in patients with STEMI did not result in any signs of myocardial damage. In addition, there was no biochemical or electrocardiographic evidence of myocardial ischemia during the G-CSF treatment in the present trial (data not shown). These issues are of importance considering several ongoing trials with intravenous delivery of mesenchymal stem cells in the treatment of STEMI, such as the Provacel Clinical trial (ClinicalTrials.gov: NCT00114452). One study previously observed higher rates of restenosis in patients treated with G-CSF when injected before primary percutaneous coronary intervention. In contrast, several recent clinical trials report occurrences of restenosis within the expected range and intravascular ultrasound demonstrated no increased neointima formation after G-CSF treatment. A potential limitation of this study is the exploratory nature of the analyses, which warrants for caution in the interpretation. Especially the lack of statistical significant association between CD34+ cells and systolic recovery could be attributable to low power. Also, it is important to recognize that differences in patient characteristics (eg, age, level of inflammation, infarct mass, and success of revascularization) between patients included in G-CSF trial and patients included in trials with intracoronary infusion could contribute to the differences in results.

Nevertheless, human data regarding stem cell treatment of ischemic heart disease are sparse and potentially clinically important aspects should be identified for further human or laboratory exploration. In addition, detailed descriptions of the cells used for therapy should be supplied in published trials to promote interstudy comparisons.

The identified dissociated G-CSF mobilization pattern for circulating CD34+ and mesenchymal stem cells might explain the neutral clinical effect of G-CSF treatment soon after a STEMI. The inverse association between circulating putative mesenchymal stem cells and subsequent recovery of left ventricular ejection fraction is of potential importance for ongoing and future trials with mesenchymal stem cells and should be investigated further.

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Disclosures

None.

References


