Outcomes and Risks of Granulocyte Colony-Stimulating Factor in Patients With Coronary Artery Disease

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OBJECTIVES Cytokine mobilization of progenitor cells from bone marrow may promote myocardial neovascularization with relief of ischemia.

BACKGROUND Patients with coronary artery disease (CAD) have low numbers of endothelial progenitor cells compared with healthy subjects.

METHODS Granulocyte colony-stimulating factor (G-CSF), 10 μg/kg/day for five days, was administered to 16 CAD patients. Progenitor cells were measured by flow cytometry; ischemia was assessed by exercise stress testing and by dobutamine stress cardiac magnetic resonance imaging.

RESULTS Granulocyte colony-stimulating factor increased CD34+/CD133+ cells in the circulation from 1.5 ± 0.2 μl to 52.4 ± 10.4 μl (p < 0.001), similar to the response observed in 15 healthy subjects (75.1 ± 12.6 μl, p = 0.173). Indices of platelet and coagulation activation were not changed by treatment, but C-reactive protein increased from 4.5 ± 1.3 mg/l to 8.6 ± 1.3 mg/l (p = 0.017). Two patients experienced serious adverse events: 1) non-ST-segment elevation myocardial infarction (MI) 8 h after the fifth G-CSF dose, and 2) MI and death 17 days after treatment. At 1 month after treatment, there was no improvement from baseline values (i.e., reduction) in wall motion score (from 25.7 ± 2.1 to 28.3 ± 1.9, p = 0.196) or segments with abnormal perfusion (7.6 ± 1.1 to 7.7 ± 1.1, p = 0.916) and a trend towards a greater number of ischemic segments (from 4.5 ± 0.6 to 6.1 ± 1.0, p = 0.068). There was no improvement in exercise duration at 1 month (p = 0.37) or at 3 months (p = 0.98) versus baseline.

CONCLUSIONS Granulocyte colony-stimulating factor administration to CAD patients mobilizes cells with endothelial progenitor potential from bone marrow, but without objective evidence of cardiac benefit and with the potential for adverse outcomes in some patients. (J Am Coll Cardiol 2005;46:1643–8) © 2005 by the American College of Cardiology Foundation

Vascular progenitor cells circulate in the bloodstream, with the potential for cardiovascular repair following injury (1). Identification and characterization of these cells in humans are controversial; however, one lineage derives from a subset of CD34+ hematopoietic stem cells that expresses one of the receptors for vascular endothelial growth factor (VEGF-2), with the cell surface marker CD133 further delineating cells with endothelial potential, and specifically referred to as endothelial precursor or progenitor cells (EPCs) (2–4). Endothelial precursor cells circulate in small numbers in healthy individuals (<0.01% of mononuclear cells), and it is possible that coronary artery disease (CAD) patients, who frequently have comorbidities and require multiple medications for management, have even lower numbers of EPCs in the circulation because of impaired production within bone marrow or reduced survival (5–7). Accordingly, stimulation of EPC release into the circulation may be an effective strategy for vascular repair in patients with advanced CAD in whom conventional treatments have failed. The purpose of our study was to test the safety and effectiveness of a cytokine commonly used to mobilize hematopoietic stem cells from bone marrow—granulocyte colony-stimulating factor (G-CSF)—in patients with CAD and chronic myocardial ischemia.

METHODS

Patients and study design. In accordance with primary eligibility criteria, all study participants were Canadian
Cardiovascular Society (CCS) class III or IV despite prior attempts at coronary revascularization (and considered unsuitable for or unwilling to undergo additional procedures) no sooner than six months before study participation, but with a stable pattern of angina for at least two months, and had reproducible myocardial ischemia by dobutamine stress cardiac magnetic resonance imaging (MRI). Medical therapy for all patients included HMG-CoA reductase inhibitors, reported previously to increase levels of EPCs in the circulation (8); all medications were maintained throughout the study. Sixteen patients (11 men and 5 women, age range 42 to 71 years) fulfilled eligibility criteria and received G-CSF (filgrastim; Amgen, Thousand Oaks, California) at baseline and within 12 h of the third, fourth, and fifth doses. CD34 and CD133+ cells with low forward and side scatter that expressed both CD45 and CD34 were counted as CD34+ cells. CD34+ cells were mobilized of progenitor cells, receiving the same G-CSF dose and treatment schedule as study participants.

**Assays.** Circulating progenitor cells were quantitated by automated cell counting (CellDyn 4000, Abbott Laboratories, Palo Alto, California) and flow cytometric phenotyping (EPICS, Beckman Coulter, Hialeah, Florida) on EDTA-anticoagulated peripheral blood samples. Each sample was diluted with HBSS (Cambrex, Walkersville, Maryland) and 0.1% human serum albumin to achieve a white blood cell count of 10^7 per tube, then incubated with human IVIgG for blocking and stained with fluorochrome-conjugated monoclonal antibodies anti-CD45-EC (Coulter), anti-CD34-FITC (Becton Dickinson, San Jose, California), and anti-AC133-PE (Miltenyi Biotec, Auburn, California). A total of 10^5 events were acquired on each of two tubes. Cells with low forward and side scatter that expressed both CD45 and CD34 were counted as CD34+ cells. CD34+ and CD133+ populations were expressed as number of circulating cells per volume of peripheral blood, based on the nucleated cell count.

Platelet factor-4 was measured by enzyme-linked immunosorbent assay (Diagnostica Stago, Asnieres, France) at baseline and within 12 h of the third, fourth, and fifth doses of G-CSF. Samples were drawn at least 2 h after the last heparin flush into vacutainer tubes containing citrate, theophylline, adenosine, and diprydamole and immediately placed on ice. Thrombin-antithrombin levels were measured by enzyme-linked immunosorbent assay (Behring Diagnostics, San Jose, California). C-reactive protein (CRP) was measured in plasma using a high-sensitivity (0.1 mg/l), two-site chemiluminescent enzyme immunometric assay (Immulite 2000, DPC, Los Angeles, California).

**Stress testing.** Demonstration of ischemia by dobutamine stress MRI was chosen for determination of eligibility and the primary end point of the study. Magnetic resonance imaging was performed on a 1.5-T clinical scanner (General Electric Medical Systems, Waukesha, Wisconsin). Five imaging planes—three short-axis (basal, mid, apical) and two long-axis (two- and four-chamber views)—were acquired at rest and at each stress stage using either a fast gradient echo (FGRE) or steady-state free precession (SSFP) technique. The imaging parameters for FGRE/SSFP were repetition time (TR) 6.2/3.6 ms, echo time (TE) 2.4/1.6 ms, flip 15/45°, 28 to 36 cm field of view, slice thickness 5/8 mm, and matrix 192 × 160. Following rest imaging, dobutamine was infused at 5 μg/kg/min and increased to 10, 20, 30, and 40 μg/kg/min in 3-min intervals. Atropine was administered at 0.25-mg increments (maximum dose 1 mg) if the heart rate remained <100 beats/min. At peak stress, a myocardial perfusion scan was also performed using gadolinium 0.1 mmol/kg given intravenously at a rate of 5 ml/s. The perfusion scan used an echo planar imaging sequence (TR 6.4 ms, TE 1.5 ms, saturation prep flip 70°, read-out flip 20°, echo train length of 4, field of view 32 to 36, matrix 128 × 96, and slice thickness 8 mm). Segmental wall motion analysis was based on the 16-segment model proposed by the American Society of Echocardiography. An ischemic response was defined as a new or worsening wall motion abnormality or a biphasic response (improvement of baseline wall motion abnormality at low dose with worsening at high dose) in one or more segments. The composite wall motion score during dobutamine stress was calculated by summing the score of each of 16 segments as normal = 0, mild = 1, moderate = 2, or severe = 3, and the score for each segment was summed to give a composite perfusion score for each study.

As a secondary end point, symptom-limited exercise testing was conducted using the modified Bruce protocol.

**Statistical analysis.** The primary efficacy end point was prespecified as ≥2-point improvement in MRI-determined regional left ventricular contractile pattern (i.e., ≥2-point reduction in composite wall motion score) during dobutamine stress at one month following G-CSF treatment compared with baseline measurements. Because of the absence of treatment benefit as assessed by treadmill exercise duration and the occurrence of two serious adverse events to be
described, enrollment was stopped early after 16 patients rather than the intended 36 and all data were analyzed. Data were analyzed by Student paired $t$ test and by repeated-measures analysis of variance (nonparametric Mann-Whitney test for CRP), with post-hoc Student-Newman-Keuls testing when statistical significance was demonstrated. Data are reported as mean $\pm$ SEM.

**RESULTS**

As shown in Table 1, most patients reported musculoskeletal pain, especially in the hips, sternum, and back, which responded to acetaminophen in all cases. Six patients reported anginal chest pain; four requested nitroglycerin. As expected, lactate dehydrogenase, alkaline phosphatase, and uric acid values increased significantly following G-CSF administration; no other values on the 20-panel chemistry panel were significantly altered by treatment.

**Cellular responses to G-CSF.** Granulocyte colony-stimulating factor increased white blood cells nearly fivefold, of which 80% to 90% were polymorphonuclear leukocytes, from baseline values at 6 to 24 h after the fifth dose of G-CSF (Table 1). CD34+ cells in sampled patients increased from $1.9 \pm 0.2 \times 10^3/\mu l$ at baseline to $55.7 \pm 11.1 \times 10^3/\mu l$ after G-CSF (Fig. 1); healthy control patients increased from $2.5 \pm 0.3 \times 10^3/\mu l$ to $84.3 \pm 13.3 \times 10^3/\mu l$ ($p = 0.118$, $p = 0.108$, respectively, vs. sampled patients). CD34+/CD133+ cells in patients increased from $1.5 \pm 0.2 \times 10^3/\mu l$ at baseline to $52.4 \pm 10.4 \times 10^3/\mu l$ after G-CSF; healthy controls increased from $1.9 \pm 0.3$ to $75.1 \pm 12.6 \times 10^3/\mu l$ ($p = 0.123$ and $p = 0.173$, respectively, vs. patients). Variability in CD34+ and in CD34+/CD133+ mobilization responses were apparent in our study participants as well as in healthy controls. One week later, CD34+ and CD34+/CD133+ cells in blood were not significantly different from baseline values. Indices of platelet activation and coagulation activation were not changed by treatment, but CRP levels increased from $4.5 \pm 1.3 \text{mg/l}$ to $8.6 \pm 1.3 \text{mg/l}$ ($p = 0.017$) at 6 to 24 h following the fifth dose of G-CSF (Fig. 2).

**G-CSF and inducible ischemia.** At one month after treatment, 12 of 15 evaluable patients reported improvement in symptoms ($\geq 1$ CCS functional class). Cardiac MRI showed no change in resting left ventricular ejection fraction as a result of treatment (Table 2). Dobutamine infusion provoked chest pain in all patients at baseline and at the one-month after-treatment study at identical average peak doses and with similar systolic blood pressure-heart rate products. There was a trend toward a greater number of ischemic left ventricular wall segments at one month post-treatment compared with baseline segmental responses to dobutamine, and there was no improvement in the composite wall motion score, in the number of abnormally perfused segments, or in the composite perfusion score during dobutamine stress as a result of G-CSF treatment. Having observed a $2.6 \pm 1.9$-point increase in the composite wall motion score with 15 subjects, the set of plausible values for the true but unknown change in wall motion score is given by the interval $(-1.5, 6.7)$ with probability 0.95 (95% confidence). Hence, we have excluded a $\geq 2$-point decrease (i.e., improvement consistent with reduced ischemia) in the wall motion score with a probability $>0.95$.

Treadmill exercise testing during all time points of the study was accompanied by chest pain in all patients. Isch
emic ST-segment responses were difficult to assess because of resting electrocardiogram (ECG) abnormalities in most patients. There was no improvement, however, in treadmill exercise duration (modified Bruce protocol) or systolic blood pressure-heart rate product at 1 month, and in 12 patients at 3 months, compared with baseline measurements (Fig. 3).

**Serious adverse events.** Two serious adverse events were encountered during our study. The first was a 52-year-old adult-onset diabetic man with a history of two myocardial infarctions and CCS functional class III angina despite previous percutaneous coronary intervention. Cardiac MRI showed resting anteroseptal and inferior hypokinesis of the left ventricle with an ejection fraction of 40%. With dobutamine infusion, new or worsening hypokinesis was noted in the lateral, mid-septum, and mid-inferior walls. Eight hours after the fifth dose of G-CSF, he complained of severe chest pain with nausea and diaphoresis. Electrocardiogram showed new ST-segment depression in leads I and aVL and mild ST-segment elevation with T-wave inversion in III.

**Table 2.** Cardiac MRI Hemodynamics in 15 Evaluable Patients at Baseline and at One Month Following G-CSF Treatment

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post G-CSF</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting left ventricular ejection fraction (%)</td>
<td>51.5 ± 2.6</td>
<td>51.2 ± 3.1</td>
<td>0.812</td>
</tr>
<tr>
<td>Peak dobutamine dose (µg/kg/min)</td>
<td>32.0 ± 2.2</td>
<td>31.5 ± 1.8</td>
<td>1.00</td>
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<tr>
<td>Peak pressure-rate product (x10^9)</td>
<td>20.3 ± 1.0</td>
<td>20.1 ± 0.7</td>
<td>0.951</td>
</tr>
<tr>
<td>Ischemic segments (n)</td>
<td>4.5 ± 0.6</td>
<td>6.1 ± 1.0</td>
<td>0.068</td>
</tr>
<tr>
<td>Wall motion score</td>
<td>25.7 ± 2.1</td>
<td>28.3 ± 1.9</td>
<td>0.196</td>
</tr>
<tr>
<td>Segments with abnormal perfusion (n)</td>
<td>7.6 ± 1.1</td>
<td>7.7 ± 1.1</td>
<td>0.916</td>
</tr>
<tr>
<td>Perfusion score</td>
<td>11.9 ± 1.8</td>
<td>12.1 ± 1.8</td>
<td>0.923</td>
</tr>
</tbody>
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Data are mean ± SEM.

G-CSF = granulocyte colony-stimulating factor; MRI = magnetic resonance imaging.
aVF. White blood cell count was $32.7 \times 10^3/\mu l$; CRP was 4.5 mg/l. Troponin I peaked at 4.2 ng/ml (upper limit of normal 2.0 ng/ml) and creatine kinase-MB fraction peaked at 7.1 $\mu g/l$ (upper limit of normal 5.0 $\mu g/l$). There were no new wall motion abnormalities by echocardiography. The patient was treated with enoxaparin and nitroglycerin paste; no further chest pain was reported, and ECG changes resolved over the next two days. Dobutamine stress cardiac MRI at one month after treatment showed similar findings as his pretreatment study.

The second event was experienced by a 69-year-old man with CCS functional class IV angina and a history of myocardial infarction, 16 percutaneous coronary interventions, 2 coronary bypass operations, transmyocardial laser revascularization, and 2 courses of enhanced external counterpulsation. Cardiac MRI showed resting inferior, posterior, and lateral wall motion abnormalities with an ejection fraction of 48%. New mid-posterior and apical hypokinesis was observed with dobutamine infusion. The patient experienced three episodes of angina during the treatment period without changes in his ECG. White blood cell count was $46.3 \times 10^3/\mu l$ and CRP was 13.4 mg/l after G-CSF. The patient was discharged from the Clinical Center but admitted to his local hospital two weeks later because of severe chest pain, initially with negative markers for infarction. Three days into that admission, he experienced recurrent severe chest pain with positive markers for infarction and developed electrical-mechanical dissociation followed by asystole and death. The family declined a postmortem examination.

DISCUSSION

We show in this study of patients with advanced CAD and recurrent ischemia that G-CSF, a cytokine commonly used to mobilize hematopoietic stem cells in healthy subjects for transplant purposes, increases circulating CD34+ and CD34+/CD133+ cells. Peak CD34+ and CD34+/CD133+ responses were approximately 80% of the average responses measured in our healthy control population. We have recently reported that G-CSF enhances EPC colony-forming ability and increases chemokine receptor expression that is important for progenitor cell homing and engraftment (9). Although investigators using the hindlimb ischemia model and the carotid injury model and a small clinical trial had suggested favorable effects of a cytokine mobilization approach (10–12), no objective benefit of progenitor cell mobilization was observed in our study. In this regard, our study had sufficient power to exclude a $\geq 2$-point decrease (i.e., improvement consistent with reduced ischemia) in the MRI-determined wall motion score with a probability $>0.95$. Further, we saw no improvement in treadmill exercise at one and three months following treatment. Recent animal data suggest that augmentation of vascular progenitor cells in the circulation fail to revascularize ischemic myocardium unless accompanied by enhanced expression of homing ligands VEGF-2 and SDF-1, respectively (13,14).

Granulocyte colony-stimulating factor-induced increases in CRP, previously reported in healthy subjects (15), may have contributed to two serious adverse events in our study. Sporadic cases of myocardial infarction have also been reported in cancer patients and in healthy subjects receiving G-CSF (16–21). In vitro experiments have shown that pro-inflammatory effects of CRP (22,23) might provoke plaque destabilization and rupture in some CAD patients. We are aware of one other study in which CRP levels were measured in CAD patients following G-CSF administration. Kang et al. (24) reported that G-CSF did not elevate levels of CRP in their patients, but that may have resulted from inclusion of patients with recent MI, in whom marked increases in CRP as a result of myonecrosis might have masked any G-CSF–related effects on CRP levels. In this study, as well as that of Seiler et al. (12), in which granulocyte macrophage-CSF was administered to CAD patients, no acute adverse effects of cytokine administration were reported.

Our findings establish that G-CSF administration to CAD patients mobilizes CD34+/CD133+ progenitor cells into the circulation, albeit to levels somewhat less than in healthy subjects. We found no objective evidence of cardiac benefit, however, which may indicate that too few cells with neovascularization potential localize to ischemic tissue for measurable efficacy. An alternative explanation is that the mobilized bone marrow derived cells are functionally impaired with a reduced neovascularization capacity (25). However, this approach may still have clinical utility for the purpose of leukapheresis with administration of selected or modified cells directly into myocardium. Significant increases in inflammatory cells and CRP, however, may contribute to adverse outcomes during treatment in some patients.

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REFERENCES


