Myocardial Salvage Through Coronary Collateral Growth by Granulocyte Colony-Stimulating Factor in Chronic Coronary Artery Disease

A Controlled Randomized Trial

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Background—The efficacy of granulocyte colony-stimulating factor (G-CSF) for coronary collateral growth promotion and thus impending myocardial salvage has not been studied so far, to our best knowledge.

Methods and Results—In 52 patients with chronic stable coronary artery disease, age 62±11 years, the effect on a marker of myocardial infarct size (ECG ST segment elevation) and on quantitative collateral function during a 1-minute coronary balloon occlusion was tested in a randomized, placebo-controlled, double-blind fashion. The study protocol before coronary intervention consisted of occlusive surface and intracoronary lead ECG recording as well as collateral flow index (CFI, no unit) measurement in a stenotic and a normal coronary artery before and after a 2-week period with subcutaneous G-CSF (10 μg/kg; n=26) or placebo (n=26). The CFI was determined by simultaneous measurement of mean aortic, distal coronary occlusive, and central venous pressure. The ECG ST segment elevation disappeared significantly more often in response to G-CSF (11/53 vessels; 21%) than to placebo (0/55 vessels; P=0.0005), and simultaneously, CFI changed from 0.121±0.087 at baseline to 0.166±0.086 at follow-up in the G-CSF group, and from 0.152±0.082 to 0.131±0.071 in the placebo group (P<0.0001 for interaction of treatment and time). The absolute change in CFI from baseline to follow-up amounted to +0.049±0.062 in the G-CSF group and to −0.010±0.060 in the placebo group (P<0.0001).

Conclusions—Subcutaneous G-CSF is efficacious during a short-term protocol in improving signs of myocardial salvage by coronary collateral growth promotion. (Circulation. 2009;120:1355-1363.)

Key Words: arteries ■ coronary circulation ■ collateral circulation ■ granulocyte colony-stimulating factor

In patients with coronary artery disease (CAD), the size of myocardial infarct size (ECG ST segment elevation) and on quantitative collateral function during a 1-minute coronary balloon occlusion was tested in a randomized, placebo-controlled, double-blind fashion. The study protocol before coronary intervention consisted of occlusive surface and intracoronary lead ECG recording as well as collateral flow index (CFI, no unit) measurement in a stenotic and a normal coronary artery before and after a 2-week period with subcutaneous G-CSF (10 μg/kg; n=26) or placebo (n=26). The CFI was determined by simultaneous measurement of mean aortic, distal coronary occlusive, and central venous pressure. The ECG ST segment elevation disappeared significantly more often in response to G-CSF (11/53 vessels; 21%) than to placebo (0/55 vessels; P=0.0005), and simultaneously, CFI changed from 0.121±0.087 at baseline to 0.166±0.086 at follow-up in the G-CSF group, and from 0.152±0.082 to 0.131±0.071 in the placebo group (P<0.0001 for interaction of treatment and time). The absolute change in CFI from baseline to follow-up amounted to +0.049±0.062 in the G-CSF group and to −0.010±0.060 in the placebo group (P<0.0001).

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Methods

Patients
Fifty-two patients (age 62±10 years, 46 men) with chronic stable 1- (n=11), 2- (n=22), or 3-vessel (n=19) CAD eligible for percutaneous coronary intervention (PCI) of at least 1 stenotic lesion were included in the study. All underwent diagnostic coronary angiography because of symptoms related to CAD. Patients were selected on the basis of the following criteria: (1) no previous transmural infarction in the myocardial areas assessed for coronary collaterals, (2) normal left ventricular ejection fraction, (3) no conge
tive heart failure, (4) no baseline ECG ST segment abnormalities, (5) no signs of inflammatory illness, (6) absence of overt neoplastic disease, and (7) no diabetic retinopathy. Patients were randomly assigned to a 2-week, double-blind protocol of subcutaneous G-CSF (filgrastim, Neupogen; Amgen Inc, Thousand Oaks, Calif; n=26) or placebo (n=26). Subcutaneous injections were performed by a study nurse not involved in the process of data acquisition and analysis. The ECG signs of ischemia and collaterals were assessed during balloon occlusion in a stenotic and, if possible, in an angiographically and functionally normal coronary artery at baseline before and immediately after the treatment period. This investigation was approved by the institutional ethics committee, and the patients gave written informed consent to participate in the study.

Cardiac Catheterization and Coronary Angiography
Patients underwent left heart catheterization for diagnostic purposes from the right femoral approach. Aortic pressure was measured using a 6F PCI guiding catheter. Central venous pressure was obtained via the right femoral vein. Left ventricular end-diastolic pressure was determined during vessel patency. Biplane left ventriculography was performed, followed by biplane coronary angiography. Coronary artery stenoses were determined quantitatively as percent diameter narrowing.

Invasive Coronary Assessment

Primary Study End Points
Signs of myocardial ischemia were assessed dichotomously according to the presence or absence of ECG signs of myocardial ischemia at the end of a 1-minute balloon occlusion of the vessel of interest. Myocardial ischemia indicative of potential future infarct size was defined as ST segment elevation >0.1 mV present on any of 4 surface leads or on an intracoronary ECG lead obtained from the angioplasty guide wire via a cross-clamp to lead V1.2,6,7 Coronary collateral flow relative to normal antegrade flow through the nonoccluded coronary artery (collateral flow index [CFI]) was determined using coronary pressure measurements. A 0.014-inch pressure monitoring angioplasty guide wire (Pressure Wire, Radii, Uppsala, Sweden) was set at zero, calibrated, advanced through the guiding catheter, and positioned in the distal part of the vessel of interest. The CFI was determined by simultaneous measurement of mean aortic pressure (Pao, mm Hg), the distal coronary artery pressure during balloon occlusion (Pcor, mm Hg), and the central venous pressure (CVP, mm Hg) as obtained at the end of the 1-minute occlusion. The CFI was calculated as (Pcor−CVP)/(Pao−CVP).8−10 The accuracy of pressure-derived CFI measurements in comparison with ECG signs of myocardial ischemia during occlusion and with absolute myocardial perfusion measurements has been documented previously.2,10,11

Secondary Study End Points
Myocardial ischemia during the 1-minute coronary occlusion was also characterized by the presence or absence of angina pectoris. Absolute myocardial perfusion or blood flow at rest and during hyperemia was assessed quantitatively using myocardial contrast echocardiography, whereby a previously described and validated algorithm was employed.12 Briefly, for the calculation of absolute blood flow, the constituent factors relative myocardial blood volume rBV and its refill rate β after the destruction of echo contrast microbubbles were obtained during vessel patency. Myocardial blood flow is equal to the product of rBV and β divided by myocardial tissue density.12

Study Protocol
During the treatment period, side effects related to the study medication were recorded every second day by the study nurse during a personal visit at the patient’s home. At the start of both baseline and follow-up invasive procedures, all patients received 5000 U of heparin intravenously. After diagnostic examinations, 2 puffs of oral isosorbide dinitrate were given. The coronary artery thought to be the culprit lesion responsible for the patient’s symp
toms was selected for CFI measurements. This vessel would undergo PCI after the 2-week study protocol. Additionally, an angiographically and functionally normal coronary artery was selected for CFI measurement. In both arteries, fractional flow reserve was determined for functional assessment with the pressure guide wire positioned distally in the vessel using a bolus of intracoronary adenosine (12 μg for the right, 18 μg in the left coronary artery) for induction of hyperemia. At baseline and follow-up, an adequately sized angioplasty balloon catheter was positioned proximal to the stenosis to be dilated, and at a proximal location in the normal vessel, whereas the pressure guide wire was positioned distally in the respective vessels. Balloon inflation for collateral measurement before injection of the study drug occurred in the proximal nonste
tenotic vessel segment at a pressure of 1 to 2 atmospheres. During this vessel occlusion, simultaneous Pcor, Pao, and CVP were obtained for the calculation of CFI. During the entire procedure, an intracoronary ECG obtained from the guide wire and a 4-lead surface ECG were recorded. The initial invasive procedure was followed by a 2-week out-of-hospital period with subcutaneous injections of randomly assigned G-CSF (10 μg/kg or 0.27 mL aqua ad injection) or placebo (0.1% albumin in 0.27 mL aqua ad injection) every other day starting the day after the baseline procedure. The study drug was prepared by the hospital pharmacy. The investigators were blinded to the study medication. All drugs were left unaltered during the study period. The invasive follow-up examination immediately after the treat
tment period consisted of intracoronary measurements identical with those described above. The PCI of the stenotic lesion initially selected to be dilated was performed immediately after the follow-up measurements.

Absolute myocardial blood flow at rest and during hyperemia in the areas supplied by the coronary arteries of interest was obtained using contrast echocardiography at baseline after the invasive pro
cedure, 14 days of follow-up before the invasive procedure with PCI and 6 months after study inclusion. Hyperemia was induced by intravenous adenosine (140 μg · min−1 · kg−1), and myocardial perfusion reserve was calculated as absolute blood flow during hyperemia divided by blood flow at rest (both in mL · min−1 · g−1).

Statistical Analysis
All continuous data are given as mean±SD. Baseline characteristics between the groups were analyzed by Student t tests for continuous data and by χ2/Fisher exact tests for categorical data. Outcomes at follow-up—examination of categorical variables (ECG ST-segment elevation >0.1 mV during vessel occlusion and angina pectoris) and frequency of side effects induced by the study drug were analyzed using the χ2 test. In order to achieve high robustness of statistical testing (even though data were approximately normally distributed), a nonparametric analysis of variance for longitudinal data was performed for all continuous data using a 2-factorial design with the factors treatment (G-CSF versus placebo) and time (baseline versus follow-up measurements. This test also accounts for intrasubject correlation between multiple measurements per patient (clustered data). Calculated P values correspond to the interaction of treatment and time. As a second step, within-group analyses at different time points of myocardial perfusion data were performed by a paired Student t test. Differences were considered statistically significant at a 2-sided P value of <0.05.
Results

Patient Characteristics and Clinical Data at Baseline

There were no statistically significant differences between the 2 groups relative to patients' age, sex, duration of angina pectoris, and history of myocardial infarction in a remote vascular area, nor were there statistical differences in the frequency of cardiovascular risk factors and (except for nitrates) the use of acetylsalicylic acid, clopidogrel, vasoactive drugs, statins, or diuretics (Table 1).

Invasive and Hemodynamic Data at Baseline

Except for systolic blood pressure, invasively obtained hemodynamic parameters at baseline such as heart rate, diastolic blood pressure, left ventricular ejection fraction, and left ventricular end-diastolic pressure did not differ between the groups (Table 2). At baseline, the severity of CAD and the severity of the stenotic lesion to be treated by PCI were similar among the groups. The stenotic and the nonstenotic vessels undergoing CFI measurement as well as the CFI measurement site were similarly distributed between the groups. Fractional flow reserve values of the normal versus the stenotic vessel undergoing CFI measurement were not statistically different between the groups (Table 2). However, myocardial perfusion reserve obtained by contrast echocardiography in the normal vessel was 1.95±0.81 in the G-CSF group and 2.85±1.21 in the placebo group (P=0.018), and in the stenotic vessel it was 1.83±0.74 in the G-CSF group and 2.29±1.28 in the placebo group (P=0.14). The occurrence of angina pectoris and of ECG ST-segment elevation >0.1 mV during the 1-minute coronary balloon occlusion in either the normal or the stenotic vessel was not statistically different between the groups. The CFI values in the normal and stenotic vessel were similar between the groups (Table 2).

Treatment-Induced Changes of Study End Points

Primary Study End Points

At follow-up, ECG ST-segment elevation >0.1 mV during coronary occlusion had disappeared more frequently in response to G-CSF (11/53; 21%) than to placebo (0/55; P=0.0005; Figure 1). Overall, P_{occl} increased in the G-CSF group, whereas it decreased in the placebo group, and P_{ao} decreased in both groups (Figure 2). The CFI values as obtained in 108 normal and stenotic vessels changed from 0.121±0.087 at baseline to 0.166±0.086 at follow-up in the G-CSF group and from 0.152±0.082 to 0.131±0.071 in the placebo group (P<0.0001 for interaction of treatment and time). The CFI increased significantly in the G-CSF group in
both normal and stenotic vessels \( (P=0.007 \text{ for interaction of both treatment and time}; \text{Figure 3}) \), and it remained statistically unchanged in the placebo group in both measurement site subgroups. The absolute change in CFI from baseline to follow-up as obtained in all vessels amounted to \( +0.049\pm0.062 \) in the G-CSF group and to \( -0.010\pm0.060 \) in the placebo group \( (P<0.0001 \text{ Figure 4}) \).

**Secondary Study End Points**

Angina pectoris during the 1-minute coronary balloon occlusion had disappeared significantly more often in response to G-CSF \( (6/49 \text{ vessels}; 12\%) \) than to placebo \( (0/55 \text{ vessels}; \ P=0.029) \). Absolute myocardial perfusion at rest remained unaltered in both groups, and during hyperemia it changed significantly between baseline and day 14 in normal vessels of patients treated with G-CSF \( (P=0.048 \text{ for interaction of treatment and time}) \). In normal vessels, perfusion reserve changed between baseline, 14 days, and 6 months in the G-CSF versus the placebo group \( (P=0.050 \text{ for interaction of treatment and time}) \), but it did not change between groups in stenotic vessels undergoing PCI after the measurement at 14 days \( (P=0.19 \text{ for interaction of treatment and time}; \text{Figure 6}) \). The statistically most relevant increase in perfusion reserve occurred in

![Figure 1. Frequency of conversion of ECG ST-segment elevation (ste) during study follow-up from present to absent ste (disappearance) and vice versa (ste appearance) in response to G-CSF (left) and to placebo (right).](image)

![Figure 2. Individual changes (thin lines) of mean \( P_{\text{occl}} \) (top) and mean \( P_{\text{ao}} \) (bottom) from baseline to follow-up measurement in response to G-CSF (left) and to placebo (right).](image)
normal vessels of the G-CSF group between baseline and 14 days of follow-up, whereas the respective increase in stenotic vessels before PCI did not reach statistical significance (Figure 6).

In the G-CSF group, fractional flow reserve changed from 0.91±0.07 at baseline to 0.93±0.06 at follow-up in normal vessels ($P=0.001$) and from 0.73±0.15 at baseline to 0.80±0.11 at follow-up before PCI in stenotic vessels ($P=0.049$). In the placebo group, fractional flow reserve changed from 0.93±0.05 at baseline to 0.92±0.05 at follow-up in normal vessels ($P=0.35$) and from 0.76±0.16 at baseline to 0.81±0.11 at follow-up before PCI in stenotic vessels ($P=0.11$).

Adverse Events and Side Effects

Adverse Events

One patient in the G-CSF group experienced occlusion of the left anterior descending coronary artery (stenotic vessel) during follow-up at the site of CFI measurement, whereby extensive angiographic collateralization from the right coronary artery had preserved left ventricular systolic function. In this case, CFI in the stenotic vessel increased from 0.307 at baseline to 0.381, and it increased from 0 to 0.167 at follow-up in the normal right coronary artery. One patient in the placebo group died after completion of the follow-up measurements and PCI in the course of acute stent thrombosis of the proximal left anterior descending and proximal left circumflex coronary artery.

Side Effects

Flu-like symptoms as side effects of the study drug, such as myalgia and bone pain, occurred more frequently in the group receiving G-CSF than in the placebo group (Table 3).

Discussion

This randomized clinical study in patients with chronic CAD documents for the first time, as far as we are aware, that G-CSF improves a marker of myocardial salvage by augmenting collateral function to a briefly occluded vessel. Also, G-CSF–induced improvement of myocardial perfusion re-
serve in a region subtended by a normal coronary artery is maintained for as long as 6 months.

Myocardial Salvage Versus Regeneration

One of the study’s primary end points that was positively influenced by G-CSF was myocardial salvage in case of a future coronary occlusion (ie, ECG ST-segment elevation during the first 1-minute artificial occlusion. It disappeared in 20% of patients receiving G-CSF and in none receiving placebo. In the context of G-CSF treatment with recruitment of progenitor cells, the therapeutic concept of myocardial salvage has been implicated unduly less than that of myocardial regeneration. The treatment strategy of salvage is based on the observation that myocardial cells can be saved until, but not beyond, 6 hours of complete ischemia. Consequently, rescuing cardiac myocytes in the event of acute and permanent coronary occlusion means reperfusing the dependent ischemic area by recanalizing the blocked artery. Alternatively, collateral perfusion can be promoted before coronary occlusion takes place. Among the described candidates for myocardial salvage, augmented collateral function before and after the 2-week study period was the only parameter that was different between the 2 groups (identical time and sequence of coronary occlusion, similar ischemic areas at risk for infarction, similar heart rate; Table 2). Thus, it is likely that the improved indicator of myocardial salvage was causally related to augmented collateral function in the G-CSF group. In line with the less frequent ECG ST-segment elevation after G-CSF therapy, angina pectoris during coronary occlusion disappeared more often than in the placebo group.

Hence, the concept of future myocardial salvage by means of coronary collateral regeneration during the chronic phase of CAD is feasible. Conversely, myocardial salvage in the course of acute myocardial infarction simultaneously with collateral regeneration or arteriogenesis is impossible because the slowly growing collateral vessel meets necrotic tissue. However, the concept of myocardial regeneration or repair of acute infarction independently of salvage is principally feasible, although it is controversial whether adult hematopoietic

Figure 5. Mean values of absolute myocardial perfusion (vertical axis) at rest (left) and during hyperemia (right) taken during vessel patency in the vascular area subtended by the vessels undergoing collateral flow index measurements. All changes were nonsignificant except for that indicated by a P value. Error bars indicate SE.

Figure 6. Mean values of myocardial perfusion reserve (vertical axis). Left, myocardial perfusion reserve in the group receiving G-CSF (Xs). Right, myocardial perfusion reserve in the placebo group (triangles). Error bars indicate SE.
stem cells do transdifferentiate into cardiac tissue.\textsuperscript{14} Replacement of the thin noncontractile collagenous scar after acute myocardial infarction by viable contracting myocardium has been shown in the experimental animal model using neonatal or fetal cardiomyocytes as long as 1 week after coronary artery occlusion.\textsuperscript{15} By contrast, adult as opposed to embryonic stem cells have been shown not to incorporate into growing collateral vessels in a mouse model of hindlimb ischemia.\textsuperscript{16} They appear to promote arteriogenesis in terms of “software” (paracrine arteriogenic effects through growth factor secretion by the stem cells) rather than “hardware” supply. Accordingly, in a meta-analysis that included 999 patients treated with bone marrow–derived cells for ischemic heart disease (12 studies in the setting of acute myocardial infarction, 6 in subacute infarction or chronic CAD), bone marrow transplantation in comparison with the control group improved left ventricular ejection fraction by 3.66%, it reduced infarct scar size by 5.49%, and the left ventricular end-systolic volume was diminished by 4.8 mL.\textsuperscript{17} Whether the mechanism leading to this very modest benefit is actual myocardial regeneration in the biological sense of the word remains unresolved. Alternatively and purely on physical grounds, the thickened scar tissue (by the cellular infiltrate) reduces ventricular preload and afterload on the basis of the Laplace law and thus explains the increase in ejection fraction, which is well known to be inversely related to afterload. The reduction in end-systolic volume is mathematically tied to the augmented ejection fraction and is thus not an independent finding. Additionally, the diminished scar size could result from paracrine and not from the regenerative effects of bone marrow–derived cells, which secrete cytokines, proteases, and growth factors, thereby enhancing myocyte survival.\textsuperscript{16}

### Myocardial Regeneration by G-CSF: Data From the Literature

Another approach than autologous bone marrow cell transplantation to regenerate myocardial scar tissue respectively to salvage still viable myocardium is to recruit the stem cells noninvasively to the site of injury. The concept in the setting of acute myocardial infarction is that various cytokines and growth factors such as G-CSF recruit bone marrow–derived progenitor cells that then target damaged tissue. In the present study, the peak level of CD34\textsuperscript{+} cells as obtained at day 7 after the study began amounted to $15 \pm 11 \times 10^3$/mL in the G-CSF group and to $4 \pm 3 \times 10^3$/mL in the placebo group ($P<0.0001$; data not shown), a level that was lower by a factor of 2 to 5 in comparison with previous G-CSF studies in the setting of acute myocardial infarction.\textsuperscript{18} A meta-analysis by Zohlnhöfer et al\textsuperscript{19} of 10 randomized clinical trials using stem cell mobilization by G-CSF in 445 patients with acute myocardial infarction found only an insignificant increase in left ventricular ejection fraction, which was on average 1.3% larger in the G-CSF group than in the placebo group ($P=0.36$); in addition, a more pronounced infarct size reduction was observed in the G-CSF than in the placebo group by 0.15% ($P=0.17$). A few studies, all of them nonrandomized, have tested G-CSF in patients with severe chronic CAD and, on average, have found no effect on left ventricular ejection fraction or on myocardial perfusion after a mean follow-up of 5 months.\textsuperscript{18} Relative to the above-discussed conceptual issue of myocardial salvage versus regeneration, these uncontrolled investigations are comparable with the present one. They have used a higher-dose regimen (mostly 10 µg/day) with a shorter duration of therapy (5 to 6 days), and the follow-up period has been similarly long. However, the actual study had a randomized design, and it directly obtained pathophysiological and statistically meaningful end points. To choose left ventricular ejection fraction and qualitatively assessed myocardial perfusion as end points in a study with a patient number ranging between 5 and 32 is statistically unreasonable, considering the large measurement variability of those parameters.\textsuperscript{18} In the context of earlier studies by our laboratory using colony stimulating factors and direct quantitative measurements of collateral function,\textsuperscript{20,21} the number of patients necessary to allow the detection of a $\approx 0.05$ difference in CFI between the groups or an increase of 30% in the G-CSF group was estimated to be 50. The only variable directly comparable between the studies just mentioned and the present study is myocardial perfusion, which remained unchanged in response to G-CSF after 5 months in the uncontrolled trials and which increased in the nonstenotic vascular area in the present study (maintenance of initial increase after 2 weeks of G-CSF treatment). Of course, the increase in myocardial perfusion reserve in the area subtended by the PCI-treated vessel cannot be attributed to G-CSF but to the PCI (Figure 5). The present observation of an enhanced hyperemic absolute myocardial perfusion in normal vessel regions of patients treated with G-CSF rests on the reliability of quantitative myocardial contrast echocardiography as performed in our study. In extensive validation studies incorporating direct comparison of the technique with a flow phantom, positron emission tomography, invasive Doppler measurements in CAD patients,\textsuperscript{12} and quantitative coronary angiography,\textsuperscript{22} the method has been shown to be very reliable in obtaining a wide range of absolute flow values between 0.1 and 8 mL \cdot min\textsuperscript{−1} \cdot g\textsuperscript{−1} and in detecting coronary artery stenotic lesions with a reduction >50% in diameter.

### Pharmacological Arteriogenesis in Humans

So far, the role of G-CSF in arteriogenesis has been investigated in only 1 experimental study.\textsuperscript{3} In this mouse model of acute myocardial infarction, G-CSF application resulted in a significant increase of circulating mononuclear cells expressing stem cell markers. Arterioles in the border zone of the infarcted

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**Table 3. Side Effects**

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>G-CSF (n=26)</th>
<th>Placebo (n=26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaise/fatigue, n (%)</td>
<td>12 (46)</td>
<td>13 (50)</td>
<td>0.78</td>
</tr>
<tr>
<td>Myalgia, n (%)</td>
<td>18 (69)</td>
<td>3 (12)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bone pain, n (%)</td>
<td>12 (46)</td>
<td>3 (12)</td>
<td>0.006</td>
</tr>
<tr>
<td>Headache, n (%)</td>
<td>6 (23)</td>
<td>3 (12)</td>
<td>0.27</td>
</tr>
<tr>
<td>Diarrhea, n (%)</td>
<td>3 (12)</td>
<td>5 (19)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Adverse events with total frequency <5 per group: fever, loss of appetite, nausea, abdominal pain, constipation, mucositis, insomnia, pruritus, exanthema.
myocardium showed an increased expression of intercellular adhesion molecule-1 accompanied by an accumulation of bone marrow-derived cells and proliferation of endothelial and smooth muscle cells. Histological examination of mice treated with G-CSF revealed a lower amount of granulation tissue associated with a subsequent reduction in left ventricular wall thinning and scar extension. For obvious reasons, our clinical study did not provide structural data indicative of G-CSF–induced arteriogenesis. But it documented for the first time, as far as we are aware, the efficacy of G-CSF with regard to the function of collateral vessels. Aside from G-CSF, there have been well-investigated substances with arteriogenic efficacy such as monocyte chemoattractant protein-1; another colony-stimulating factor, granulocyte-macrophage colony-stimulating factor (GM-CSF); transforming growth factor β1; fibroblast growth factors; tumor necrosis factor α; and matrix metalloproteinases. Among these, only colony-stimulating factors have been investigated for clinical use in coronary or peripheral artery disease, whereby GM-CSF has been shown to be effective in the former but not the latter condition. However, inherent to all the arteriogenic substances is a certain proatherogenic risk because of the shared pathogenic monocyte involvement and inflammation in both processes. For example, although it is a strong arteriogenic factor, monocyte chemoattractant protein-1 has been shown to be not suitable for clinical use because of its atherogenic side effects. A small randomized clinical trial using GM-CSF for arteriogenesis had to be stopped prematurely for safety concerns in the context of 2 patients with acute coronary syndrome in the GM-CSF group. By contrast, G-CSF has been reported in meta-analyses to be safe, finding that is consistent with the present data.

Study Limitations
In the context of the above-discussed aspects of the safety and side effects of G-CSF, the issue of pseudo-blinding to the study drug can be raised because it occurs in numerous “double-blind” clinical trials with distinctive study drug side effects. In the present study, and in order to account for this potential pitfall, patient visits during the study period with recording of drug side effects were performed by a study nurse not involved in data acquisition and analysis.

One of the primary study end points, ECG ST-segment elevation during the 1-minute coronary occlusion, is a surrogate marker for infarct size and not the actual amount of necrotic myocardium as obtained, for example, by technetium scintigraphy or gadolinium magnetic resonance imaging. Methodologically, it was not feasible in this study involving patients with chronic CAD to directly measure infarct size because of the absence of such an event. Also, the decrease or disappearance of ECG signs of ischemia during a brief coronary occlusion in response to certain therapeutic procedures has long been recognized as a direct measure of myocardial salvage in case of a future permanent coronary occlusion.2,31

Conclusion
Subcutaneous G-CSF is efficacious during a short-term protocol in improving signs of myocardial salvage by coronary collateral growth promotion.

Sources of Funding
This study was supported by a grant from the Swiss National Science Foundation (grant #3200BO-112341) and the Swiss Heart Foundation.

Disclosures
None.

References
13. Reimer KA, Jennings RB. The “wavefront phenomenon” of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest. 1979;40:633–644.


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**CLINICAL PERSPECTIVE**

In patients with symptomatic coronary artery disease, the size of myocardial infarction is one of the main determinants of outcome after such an event. Accordingly, therapeutic strategies aim to reduce cardiovascular mortality by shrinking infarct size. Infarct size is influenced directly by the duration of coronary occlusion, by ischemic area at risk for infarction, lack of collateral blood supply to the ischemic zone, absence of ischemic preconditioning before the infarct, myocardial oxygen consumption during the infarct, or some combination of these conditions. Aside from limiting the duration of coronary occlusion, the option of reducing infarct size by promoting collateral artery growth (arteriogenesis) is appealing. In patients with chronic coronary artery disease, a beneficial prognostic effect of well-developed versus poorly developed collaterals has been documented. As there are a number of patients who cannot undergo revascularization by percutaneous coronary intervention or bypass surgery, the finding of the present study that granulocyte—colony stimulating factor is efficacious in promoting collateral growth is important. In addition, granulocyte colony-stimulating factor was found to have a prolonged effect on myocardial perfusion in a vascular area subtended by a nonstenotic vessel and the drug was shown to be safe during the short-term protocol.
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_Circulation._ 2009;120:1355-1363; originally published online September 21, 2009; doi: 10.1161/CIRCULATIONAHA.109.866269

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/120/14/1355

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