Promotion of Collateral Growth by Granulocyte-Macrophage Colony-Stimulating Factor in Patients With Coronary Artery Disease

A Randomized, Double-Blind, Placebo-Controlled Study

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Background—Experimentally, activated macrophages have been documented to induce vascular proliferation.

Methods and Results—In 21 patients (age 74±9 years) with extensive coronary artery disease not eligible for coronary artery bypass surgery, the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF, Molgramostim) on quantitatively assessed collateral flow was tested in a randomized, double-blind, placebo-controlled fashion. The study protocol consisted of an invasive collateral flow index (CFI) measurement immediately before intracoronary injection of 40 μg of GM-CSF (n=10) or placebo (n=11) and after a 2-week period with subcutaneous GM-CSF (10 μg/kg) or placebo, respectively. CFI was determined by simultaneous measurement of mean aortic pressure (Pao, mm Hg), distal coronary occlusive pressure (Poccl, mm Hg; using intracoronary sensor guidewires), and central venous pressure (CVP, mm Hg): CFI=(Poccl−CVP)/(Pao−CVP). CFI, expressing collateral flow during coronary occlusion relative to normal antegrade flow during vessel patency, changed from 0.21±0.14 to 0.31±0.23 in the GM-CSF group (P<0.05) and from 0.30±0.16 to 0.23±0.11 in the placebo group (P=NS). The treatment-induced difference in CFI was +0.11±0.12 in the GM-CSF group and -0.07±0.12 in the placebo group (P=0.01). ECG signs of myocardial ischemia during coronary balloon occlusion occurred in 9 of 10 patients before and 5 of 10 patients after GM-CSF treatment (P=0.04), whereas they were observed in 5 of 11 patients before and 8 of 11 patients after placebo (P=NS).

Conclusions—This first clinical study investigating the potential of GM-CSF to improve collateral flow in patients with coronary artery disease documents its efficacy in a short-term administration protocol. (Circulation. 2001;104:2012-2017.)

Key Words: coronary disease ■ collateral circulation ■ growth substances ■ granulocyte-macrophage colony-stimulating factor

Cardiovascular diseases, in particular coronary artery disease (CAD), are the leading cause of death in adults in industrialized countries. Established options for revascularization include angioplasty and surgical bypass, both of which are not suitable in 1 of 5 to 1 of 3 patients in whom the extent of coronary atherosclerosis is especially severe. An alternative treatment strategy is therefore warranted both to control symptoms as well as to alter the course of advanced CAD. Therapeutic angiogenesis is a new and promising strategy for revascularizing ischemic myocardial tissue by formation of natural bypasses, ie, collaterals. Angiogenesis is induced by surgical or catheter-based delivery of various promotors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), angiogenic agents most often used in present clinical studies. In addition, controversy on the ability of angiogenic growth factors to promote coronary collaterals may be related to the use of endpoints for their assessment that are too blunt to discern subtle changes in collateral flow. Also, angiogenic factors may have been used that induce the formation of small, high-resistance capillaries (termed angiogenesis) rather than large interconnecting arterioles (term arteriogenesis), which are required for the salvage of myocardium in the ischemic zone. 

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presence of occlusive CAD.6 Experimentally, arteriogenesis has been shown to be induced by activated macrophages,7 lipopolysaccharide,8 monocyte chemotactic protein-1,9 tumor necrosis factor-α (TNF-α), bFGF, and also granulocyte-macrophage colony-stimulating factor (recombinant human GM-CSF; Molgramostim).10 Therefore, the purpose of this study was to test the hypothesis that short-term intracoronary and subcutaneous administration of GM-CSF improves recruitable collateral flow.

Methods

Patients
Twenty-one patients (age 75 ± 10 years, 10 men and 11 women) with two- (n = 7) to three-vessel (n = 14) CAD not eligible for or unwilling to undergo (n = 3) coronary artery bypass surgery and eligible for percutaneous transluminal coronary angioplasty (PTCA) of at least one stenotic lesion were included in the study. All underwent diagnostic coronary angiography because of symptoms related to stable CAD. Patients were prospectively selected on the basis of the following criteria: (1) no previous transmural infarction in the myocardial area assessed for coronary collaterals; (2) normal left ventricular ejection fraction; (3) no congestive 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 intracoronary followed by subcutaneous GM-CSF (Molgramostim; Novartis, n = 11) or placebo (n = 10) or placebo (n = 11).

Coronary collateral assessment
Angiographic collateral degree (0 to 3) was determined before vascular balloon occlusion. 0 indicated no filling by contrast of the distal vessel via collaterals; 1, small side branches; 2, major side branches of the main vessel filled; and 3, main epicardial vessel filled by collaterals. Furthermore, coronary collaterals 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 was defined as ST-segment changes >0.1 mV present on any of 3 surface leads or on an intracoronary ECG lead obtained from the angioplasty guidewire (Figure 1).

Primary End Point of the Study
Coronary collateral flow relative to normal antegrade flow through the nonoccluded coronary artery (CFI) was determined using coronary pressure measurements. A 0.014-inch pressure-monitoring PTCA guidewire (Pressure Wave, Endosonics) was set at zero, calibrated, advanced through the guiding catheter, and positioned in the distal part of the vessel of interest. CFI was determined by simultaneous measurement of mean aortic pressure (Pao, mm Hg), the distal coronary artery pressure during balloon occlusion (Poccl, mm Hg), and the central venous pressure (CVP, mm Hg; Figure 1). CFI was calculated as (Poccl−CVP)/(Pao−CVP).11 The accuracy of pressure compared with Doppler-derived CFI measurements and compared with ECG signs of myocardial ischemia during occlusion has been documented previously.11

Study Protocol
Baseline and follow-up examination included venous blood sampling for white blood cell count and assessment of C-reactive protein, creatinine, and serum lipids. During the treatment period, side-effects related to the study medication were recorded every second day by one of the investigators 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, two puffs of oral isosorbidedinitrate were given. A major coronary artery was chosen for intracoronary injection of GM-CSF or placebo, which was anatomically suitable for PTCA of a relevant stenotic lesion after the 2-week study protocol. At baseline, an adequately sized over-the-wire angioplasty balloon catheter (Ranger, Boston Scientific) was positioned proximal to the stenosis to be dilated, whereas the pressure guidewire was positioned distal to the stenosis. Balloon inflation for collateral measurement before injection of the study drug occurred in the proximal, nonstenotic vessel segment at a pressure of 1 to 3 atmospheres. During this

Figure 1. Determination of collateral flow in a patient receiving GM-CSF before (left) and after (right) therapy. Top, Surface (I, III, and avF) and intracoronary (i.c.) ECG lead recordings are shown. After 1 minute of vessel occlusion before GM-CSF treatment, there are marked ST-segment elevations on the i.c. ECG lead, indicating coronary collaterals insufficient to prevent myocardial ischemia. After therapy, i.c. ECG signs of myocardial ischemia have disappeared. CFI (no unit) is calculated by dividing distal i.c. mean occlusive pressure (Poccl, mm Hg) minus central venous pressure (CVP, mm Hg) by mean aortic pressure (Pao, mm Hg) minus CVP; before GM-CSF CFI amounts to (18−8)/(84−8)=0.13. CFI increases to 0.23 after GM-CSF treatment (right).
vessel occlusion, simultaneous $P_{\text{ext}}, P_{\text{int}}$, and CVP were obtained for the calculation of CFI (Figure 1). During the entire procedure, an intracoronary ECG obtained from the guidewire and a 3-lead surface ECG were recorded. After initial CFI determination, the angioplasty balloon was deflated, the pressure guidewire was removed, and, during a 2- to 3-minute, low-pressure reinflation, the study drug was injected through a millipore filter via the angioplasty catheter. It was prepared a few minutes before the first balloon inflation by the laboratory personnel according to the randomization table. The study drug consisted of 40 mg of GM-CSF in 5 mL of saline 0.9% or of 0.1% albumin in 5 mL of saline 0.9% (placebo). After intracoronary injection and removal of the balloon catheter, 10 mL of blood was obtained from the guiding catheter at the end of the procedure for injection and removal of the balloon catheter, 10 mL of blood was obtained from the guiding catheter at the end of the procedure for

Statistical Analysis

Power analysis before the study hypothesizing a relative change of CFI in the treatment group of $\geq 50\%$ (SD 0.10) at a significance level of $<0.05$ provided a sample size of 20 patients. Between-group comparisons of continuous clinical, hemodynamic, angiographic, and collateral flow data were performed by a Mann-Whitney test. A $\chi^2$ test was used for comparison of categorical variables among the two study groups. Intraintividual comparisons of baseline versus follow-up data were performed using Wilcoxon signed rank test. Linear regression analysis was performed to assess the existence of an association between TNF-$\alpha$ and CFI changes. Mean values $\pm SD$ are given. Statistical significance was defined at $P<0.05$.

Results

Patient Characteristics and Clinical Data at Baseline

There were no statistically significant differences between the two groups regarding age of the patients, sex, body mass index, heart rate, and severity and duration of angina pectoris. There were no statistical differences either in the frequency of cardiovascular risk factors or the use of acetylsalicylic acid or vasoactive drugs.

Hemodynamic, Angiographic, and Collateral Data at Baseline

Mean blood pressure during vessel occlusion, left ventricular ejection fraction, end-diastolic pressure, distal coronary occlusive pressure, and central venous pressure during coronary occlusion were similar between the study groups at baseline (Table 1). In the group receiving GM-CSF, the number of vessels affected by CAD tended to be higher than in the control group. There were no statistical differences among the groups in the total number of hemodynamically relevant stenoses in the vessel selected for injection of the study drug and for PTCA or in the severity of the treated and untreated stenoses. Qualitative and quantitative variables for the assessment of the collateral circulation were similar among the groups (Table 1).

Side Effects

A patient who had undergone the baseline examination with placebo and died 6 days later because of extensive CAD was not included in the study. All patients in the GM-CSF group
took the study medication until the follow-up invasive examination except for one who refused to continue the subcutaneous injections without specified reason. Side effects of the study medication included low fever or sweating, skin rashes at the site of injection, and, in <3 patients each, malaise, headache, nausea, paraesthesia, loss of appetite, and muscle pain. Patients in the GM-CSF group complained about any side effect in 6 of 10 instances, and those of the placebo group did so in 2 of 11 cases \( (P<0.05) \). Low-fever temperatures occurred in 3 of 10 patients in the GM-CSF group and in 1 of 11 individuals in the placebo group \( (P=NS) \). Skin rashes during treatment appeared in 7 of 11 cases in the GM-CSF group and in 2 of 11 placebo cases \( (P=0.01) \).

**Treatment-Induced Laboratory Parameters and Collateral Flow Changes**

Total leukocyte count, neutrophils, and monocytes increased significantly in the GM-CSF group, whereas they remained statistically unchanged in the placebo group (Table 2). After treatment, those parameters plus the eosinophil count were significantly higher in the GM-CSF than in the placebo group. C-reactive protein and serum creatinine tended to increase in the GM-CSF group, whereas they remained statistically unaltered in the placebo group. C-reactive protein and serum creatinine tended to increase in the GM-CSF group, whereas they remained statistically unaltered in the placebo group.

Angiographic collateral degree increased significantly after treatment appeared in 7 of 11 cases in the GM-CSF group and in 2 of 11 placebo cases \( (P=0.01) \).

### TABLE 2. Treatment-Induced Laboratory Parameter and Coronary Collateral Flow Changes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GM-CSF Before Treatment</th>
<th>GM-CSF After Treatment</th>
<th>GM-CSF P</th>
<th>Placebo Before Treatment</th>
<th>Placebo After Treatment</th>
<th>Placebo P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes (total), ( 10^9/L )</td>
<td>6.8±0.3</td>
<td>14.0±0.7</td>
<td>0.005</td>
<td>7.3±0.2</td>
<td>7.5±0.3*</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>4.3±0.4</td>
<td>10.5±0.6</td>
<td>0.005</td>
<td>5.2±0.5</td>
<td>5.6±0.7*</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.05±0.05</td>
<td>0.98±1.24</td>
<td>NS</td>
<td>0.06±0.05</td>
<td>0.12±0.19*</td>
<td>NS</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.03±0.04</td>
<td>0.06±0.09</td>
<td>NS</td>
<td>0.03±0.04</td>
<td>0.06±0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.29±0.36</td>
<td>0.79±0.54</td>
<td>0.04</td>
<td>0.39±0.22</td>
<td>0.35±0.21*</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.23±0.40</td>
<td>1.38±0.81</td>
<td>NS</td>
<td>1.70±0.64</td>
<td>1.51±0.50</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>18.3±33.7</td>
<td>35.6±28.4</td>
<td>0.05</td>
<td>7.7±9.1</td>
<td>22±41.6</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, ( \mu \text{mol/L} )</td>
<td>90±15</td>
<td>95±17</td>
<td>0.05</td>
<td>90±17</td>
<td>91±16</td>
<td>NS</td>
</tr>
<tr>
<td>Total serum cholesterol, ( \text{mmol/L} )</td>
<td>5.3±0.8</td>
<td>4.1±0.6</td>
<td>0.01</td>
<td>5.3±1.2</td>
<td>5.0±1.0*</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, ( \text{mmol/L} )</td>
<td>1.4±0.3</td>
<td>1.1±0.2</td>
<td>0.02</td>
<td>1.3±0.2</td>
<td>1.2±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-( \alpha ), pg/mL</td>
<td>13.9±32.6</td>
<td>20.3±30.0</td>
<td>0.10</td>
<td>27.0±81.6</td>
<td>3.8±5.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Collateral assessment**

- **Angiographic degree before occlusion, 0–3**
  - GM-CSF: 1.0±0.8
  - Placebo: 1.4±0.7
- **Angina pectoris during occlusion, %**
  - GM-CSF: 7 (70)
  - Placebo: 3 (30)
- **ECG ST changes >1 mm during occlusion, %**
  - GM-CSF: 9 (90)
  - Placebo: 5 (50)
- **Collateral flow index, no unit**
  - GM-CSF: 0.21±0.14
  - Placebo: 0.31±0.23

**Collateral flow index change between after and before**

- GM-CSF: +0.11±0.12
- Placebo: -0.07±0.12

\( ^a P<0.05 \) vs GM-CSF.

CRP indicates C-reactive protein; PTCA, percutaneous transluminal coronary angioplasty; and RCA, right coronary artery.

*Discussion*

This first study investigating the potential of Molgramostim (recombinant human GM-CSF, an established drug used in oncology for the last 10 years\(^{12}\)) to improve quantitatively...
determined coronary collateral flow in patients with advanced CAD documents its efficacy in a short-term administration protocol using the local intracoronary and systemic delivery routes.

**Clinical Trials on Angiogenesis**

Acidic FGF was the agent first chosen for angiogenic therapy in 40 patients undergoing coronary artery bypass surgery, whereby they received intramyocardial injections with active or heat-denatured protein in the supply area of the distal left anterior descending coronary artery.1 Twelve weeks later, patients having received the active growth factor appeared angiographically to accumulate more contrast dye than the control group. Except for one controlled phase I study using bFGF heparin-alginate microcapsules implanted surgically,13 subsequently performed phase I trials using VEGF or bFGF were designed as uncontrolled, open-label investigations.3,4,14,15 Although they all have documented safety and feasibility of VEGF and bFGF, some preliminary evidence for possible efficacy in the case of bFGF has been provided only in the mentioned controlled work by Laham et al,13 in which an improvement of regional myocardial perfusion was demonstrable by nuclear stress testing 16 months after treatment. Although the ultimate goal of angiogenic therapy in CAD is to reduce adverse cardiac events by collateralizing ischemic myocardium, presently surrogate endpoints for death, infarction, unstable angina pectoris, etc, have to suffice. Patient numbers are small related to the ongoing selection process among numerous angiogenic factor candidates. Growth factors assumed to promote collateral growth should be clearly shown to increase collateral flow. The requirement of collateral flow measurement is not fulfilled using as study endpoint noninvasively obtained myocardial perfusion during vessel patency, because respective increases can be attributable to augmented native vessel flow, collateral flow, or both. Our study used repetitive invasive measurements during vascular occlusion, which is the only possibility to reliably gauge collateral flow.

The only preliminary controlled phase II trial of a growth factor, the VEGF to Improve left Ventricular Function and Angiogenesis (VIVA) trial, has included 178 patients receiving intracoronary and intravenous VEGF165.6 Improvements in treadmill time and angina pectoris at 60 and 120 days were noted in all groups without statistical difference among patients receiving VEGF165 or placebo. The VIVA-preceding, uncontrolled phase I trial had found an improvement of resting nuclear perfusion in the high-dose group.3 Pathophysiologically, the lacking efficacy of VEGF may be interpreted on the basis of its ability to promote the growth of small rather than large, conductive collateral vessels.6,10

**Arteriogenesis and GM-CSF: Data From the Literature**

In patients with severe, advanced CAD, having experienced sufficient myocardial ischemic stimuli to incite the sprouting of small collateral vessels, their development into large, conductive collaterals is warranted to augment perfusion in the stenotic vascular area to such an extent that parameters such as exercise time or even cardiac mortality will improve. So far, promotion of large, conductive collaterals (ie, arteriogenesis⁸) has been investigated only experimentally. Monocyte chemoattractant protein-1, a regulator for monocyte trafficking to sites of inflammation, has been found to be a very potent enhancer of angiogenesis and arteriogenesis in rabbits.16 Arras et al⁹ documented a similar effect by an intravenous infusion of the endotoxin lipopolysaccharide, the strongest activator of TNF-α in monocytes and macrophages. TNF-α is responsible for adhesion and activation of additional monocytes via upregulation of cell adhesion molecules and by upregulation of GM-CSF.10 Apart from these recent investigations, there was also an early study by Polverini et al⁷ documenting in guinea pig corneas that activated macrophages induce vascular proliferation. Our clinical study provides proof of principle of the coronary arteriogenic potential of GM-CSF by verifying the hypothesis that a short-term local and systemic application protocol augments collateral flow. However, collateral flow index responses in the GM-CSF group were quite variable, including 4 good responders, 3 moderate responders, and 3 nonresponders (Figure 2). Speculation on reasons for the changeable effect of the study drug include the fact that one patient, a nonresponder, did not complete the drug administration phase, and that sedentary patients might not have experienced enough ischemic stimuli to induce angiogenesis. The observation of a direct relation between therapy-induced collateral flow augmentation and TNF-α at follow-up raises the possibility that TNF-α itself was the decisive arteriogenic factor. A reflection of the cytokine increase accompanying collateral flow augmentation in the GM-CSF group is the tendency to elevated C-reactive protein and the more frequent episodes of subfebrile temperatures than in the placebo group.

**GM-CSF and Atherogenesis**

Aside from the role of those changes as indicators of ongoing coronary arteriogenesis, they may be interpreted as a possible sign of a proatherogenetic effect of GM-CSF. Because angiogenesis may also involve vasa vasorum of atherosclerotic plaques, such an interpretation would be reasonable, and proatherogenetic effects of angiogenic factors have been documented in the case of VEGF.17 Follow-up in our study was too short to detect progression of coronary atherosclerotic lesions. The atherogenic risk factor profile altered significantly in response to GM-CSF, showing a reduction in both total and HDL cholesterol. To speculate that these changes may have even caused regression of atherosclerosis would be unjustifiable, because the cholesterol-to-HDL ratio remained unchanged. Documentation of a potential atherogenic effect of GM-CSF has been controversial. It includes experimental work by Shindo et al,18 who found a reduced surface area of atheromatous plaques in the aortic arch of hyperlipidemic rabbits treated with GM-CSF–treated versus placebo-treated animals. Conversely, recent clinical studies have revealed a direct association between atherosclerotic progression of CAD (ie, unstable angina pectoris group) and M-CSF plasma concentration, with elevated M-CSF levels even predicting future cardiac events during a 20-month follow-up.19 Because patients with unstable angina pectoris suffer more cardiac events than those with stable CAD, the
above-mentioned association may be interpreted as a sign of augmented collateral growth activity instead of atherosclerotic progression among the unstable CAD patients.

Study Limitations
Because theoretically the manifestation of side-effects would have carried a certain risk of uncovering the randomization table, the investigator recording the side-effects and the one performing collateral measurements and data analysis were different.

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References
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