Differential Effect of Intracoronary Infusion of Mobilized Peripheral Blood Stem Cells by Granulocyte Colony–Stimulating Factor on Left Ventricular Function and Remodeling in Patients With Acute Myocardial Infarction Versus Old Myocardial Infarction: The MAGIC Cell-3-DES Randomized, Controlled Trial

Hyun-Jae Kang, Hae-Young Lee, Sang-Hoon Na, Sung-A Chang, Kyung-Woo Park, Hyung-Kwan Kim, Song-Yi Kim, Ho-Joon Chang, Whal Lee, Won Jun Kang, Bon-Kwon Koo, Yong-Jin Kim, Dong Soo Lee, Dae-Won Sohn, Kyou-Sup Han, Byung-Hee Oh, Young-Bae Park and Hyo-Soo Kim

Circulation. 2006;114:I-145-I-151
doi: 10.1161/CIRCULATIONAHA.105.001107

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 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/114/1_suppl/I-145

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/
Differential Effect of Intracoronary Infusion of Mobilized Peripheral Blood Stem Cells by Granulocyte Colony–Stimulating Factor on Left Ventricular Function and Remodeling in Patients With Acute Myocardial Infarction Versus Old Myocardial Infarction

The MAGIC Cell-3-DES Randomized, Controlled Trial

Hyun-Jae Kang, MD; Hae-Young Lee, MD; Sang-Hoon Na, MD; Sung-A Chang, MD; Kyung-Woo Park, MD; Hyung-Kwan Kim, MD; Song-Yi Kim, MD; Ho-Joon Chang, MD; Whal Lee, MD; Won Jun Kang, MD; Bon-Kwon Koo, MD; Yong-Jin Kim, MD; Dong Soo Lee, MD; Dae-Won Sohn, MD; Kyou-Sup Han, MD; Byung-Hee Oh, MD; Young-Bae Park, MD; Hyo-Soo Kim, MD

Background—The efficacy of intracoronary infusion of granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSCs) has not been compared between patients with acute (AMI) versus old myocardial infarction (OMI). In addition, the potential risk of restenosis associated with G-CSF–based stem cell therapy has not been evaluated in the setting of drug eluting stent (DES) implantation.

Methods and Results—We randomly allocated 96 patients with myocardial infarction who underwent coronary revascularization with DES for the culprit lesion into 4 groups. Eighty-two patients completed 6-month follow-up; AMI cell infusion (n=25), AMI control (n=25), OMI cell infusion (n=16), and OMI control group (n=16). In cell infusion groups, PBSCs were mobilized by G-CSF for 3 days and delivered to infarcted myocardium via intracoronary infusion. The AMI cell infusion group showed a significant additive improvement in left ventricular ejection fraction (LVEF) and remodeling compared with controls (change of LVEF: +5.1±9.1% versus −0.2±8.6%, P<0.05; change of end-systolic volume: −5.4±17.0 mL versus 6.5±21.9 mL, P<0.05). In OMI patients, however, there was no significant change of LVEF and ventricular remodeling in spite of significant improvement of coronary flow reserve after cell infusion. G-CSF–based cell therapy did not aggravate neointimal growth with DES implantation.

Conclusions—Intracoronary infusion of mobilized PBSCs with G-CSF improves LVEF and remodeling in patients with AMI but is less definite in patients with OMI. G-CSF–based stem cell therapy with DES implantation is both feasible and safe, eliminating any potential for restenosis. (Circulation. 2006;114[suppl I]:I-145–I-151.)

Key Words: myocardial infarction ■ stem cell ■ G-CSF

Recent clinical studies1–6 reported favorable effects of stem cell transplantation in patients with acute myocardial infarction (AMI). However, the outcome has not been adequately evaluated in old myocardial infarction (OMI) patients. Granulocyte colony-stimulating factor (G-CSF)–based stem cell therapy has been proposed as a practical and noninvasive alternative to stem cell therapy using bone marrow stem cells. Because G-CSF alone has only shown equivocal benefits in previous clinical trials,5,8 G-CSF might be considered mostly as a mobilizer to enrich peripheral blood stem cells (PBSCs). Despite the potential adverse effects increasing vascular events,9–11 short-term use of G-CSF in patients with myocardial infarction (MI) seems safe. Previously, we reported that, in patients with MI, intracoronary infusion of PBSCs improved cardiac function and exercise capacity, whereas the administration of G-CSF alone did not.5 Additionally, we suggested the possibility of aggravated restenosis after G-CSF administration. Therefore, in the Myocardial Regeneration and Angiogenesis in Myocardial...
Infarction With G-CSF and Intra-Coronary Stem Cell Infusion-3-Drug Eluting Stents (MAGIC Cell-3-DES) Trial, we adopted the exclusive use of drug eluting stents (DES) and modified the timing of G-CSF treatment to minimize the risk of restenosis and inflammation. This trial was performed to evaluate the safety of G-CSF–based stem cell therapy and to compare outcome of intracoronary infusion of mobilized PBSCs between patients with AMI and OMI.

**Methods**

**Patients and Protocol**

The MAGIC Cell-DES trial was designed as a randomized, controlled trial to recruit 100 patients with AMI and OMI (Figure 1). In this study, AMI was defined as randomization within 14 days from onset of new ST-segment elevation infarction, and OMI was defined as a randomization later than 14 days from onset. Patients who were successfully revascularized with DES in the culprit lesion were eligible for enrollment. Exclusion criteria were: (1) persistent severe heart failure (left ventricular ejection fraction [LVEF] \(< 20\%\)); (2) uncontrolled myocardial ischemia or ventricular tachycardia; (3) culprit lesion of infarct related artery not feasible for percutaneous coronary intervention (PCI) or unsuccessful PCI; (4) age \(\geq 80\) years; (5) malignancy; (6) serious current infection or hematologic disease; and (7) life expectancy \(< 1\) year.

After revascularization, patients were randomly assigned by use of a randomization table. After randomization, study processes were not blinded. The study protocol was approved by the Institutional Review Board of Seoul National University Hospital. Informed written consents were obtained from patients after explaining the procedure and risk of the study.

The primary end point to evaluate efficacy was the change in LVEF, measured by MRI. The secondary end points were changes in left ventricle (LV) volume, myocardial perfusion measured by coronary flow reserve (CFR), and the development of major adverse cardiac events ([MACE] death, new MI, revascularization, or hospitalization because of aggravation of ischemia or heart failure). We calculated that we would need 20 patients in each group to detect a difference in global LVEF change of 7%, with an 80% power and a 2-sided significance level of \(P<0.05\). We adjusted the sample size for an estimated follow-up loss rate of 25%, which results in 25 patients in each group.

**Stem Cell Mobilization, Characterization, and Intracoronary Infusion**

In the cell infusion groups after successful PCI, PBSCs were mobilized by daily subcutaneous injections of G-CSF (Dong-A Pharmaceutical) at 10 \(\mu\)g/kg body weight for 3 days. At day 4, mobilized PBSCs were collected with COBE spectra apheresis system (COBE BCT Inc) using the mononuclear cell collection methods. The infusion cell doses were 1 to 2 \(10^9\) monocytes per patients to guarantee the minimum target cell dose of 7 \(10^6\) CD34+ cells. We evaluated the composition of mobilized cells by flow cytometry with anti-VEGFR2, anti-CD34, and anti-AC133 antibodies (Santa Cruz Biotechnology Inc). We infused PBSCs selectively to infarcted myocardium via over-the-wire balloon catheter as described previously.5 Placebo was not applied to the control group.

**Cardiac MRI**

Cardiac MRI (Sonata 1.5T, Siemens) was performed after PCI at baseline and at 6 months. Short axis cine images with a thickness of 8 mm and a gap of 2 mm were acquired throughout the entire LV using contiguous 2D steady state precession sequences. After intravenous application of gadolinium-diethylene-triamine penta-acetate, late enhancement (LE) imaging was performed with phase-sensitive inversion recovery sequence.12 Using the ARGUS software (Siemens), LVEF, LV volumes, and volume of LE were calculated.

**Angiography, CFR, and Quantitative Coronary Angiography**

Coronary angiograms were obtained at initial PCI and 6-month follow-up. Quantitative coronary angiography (QCA) was performed by an independent blinded specialist with a Quantcor QCA V4.0 program (Pie Medical Imaging). Binary restenosis was defined as a diameter stenosis \(> 50\%\) within the stented segments including...
5-mm segments from stent margin. CFR was calculated as the ratio of hyperemic to baseline coronary blood flow velocity using a Doppler guide wire (Flowire, Volcano Corp) at 1 cm distal to the implanted stent. CFR was measured at baseline and at 6-month follow-up at the same site as described previously.5

Clinical Safety and Follow-Up Visits
To study the safety of G-CSF–based stem cell therapy, the development of MACE; clinical status including G-CSF–related pain, dyspnea, and chest pain; and biochemical tests including creatine kinase (CK)-MB, C-reactive protein (CRP), and blood cell counts, were evaluated during admission and follow-up visits scheduled at 1, 3, and 6 months.

Statistical Analysis
Continuous variables were presented as mean±SD. Categorical variables were compared with the χ2 test. Statistical comparisons of continuous variables between initial and follow-up data were performed using a paired t test for comparison for intragroup comparisons and a Student’s t test for intergroup comparisons. Statistical significance was assumed at a value of P<0.05. Statistical analysis was performed with SPSS (version 13.0, SPSS Inc).

The authors had full access to the data and take responsibility for its integrity. All of the authors have read and agreed to the article as written.

Results
Since January 2004, 96 patients were enrolled and randomized into 4 groups: enrolled patients were stratified by the presence or absence of AMI and randomly assigned into the cell infusion and control group, respectively. Enrollment of the OMI group was stopped prematurely because of unlikelyhood of achieving a significant difference in the primary end point (improvement of LVEF). Among them, 82 patients completed 6-month follow-up (Figure 1). The baseline characteristics of the participants who completed the follow-up evaluation are summarized in Table 1. Clearly, the time to randomization from onset of infarction was quite different between AMI an OMI groups (7.0±1.0 versus 517±525 days).

Procedural Safety and Clinical Follow-Up
There were no serious adverse reactions related to G-CSF administration during the peri-procedural period. Six patients (14.6%) complained of transient G-CSF–related adverse effects: bone pain (n=2), headache (n=2), injection site tenderness (n=1), and dizziness (n=1). During G-CSF injection and hospitalization, we did not observe any aggravation of ischemia or thrombotic complications. Systemic inflammation as measured by CRP was not significantly different between the cell infusion and control group in the decline of CRP from baseline to day 4 in AMI (P>0.05). However, in OMI patients, G-CSF slightly but significantly increased CRP.

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Characteristics of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>Clinical parameters</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Sex (male)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
</tr>
<tr>
<td>Risk factors</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>PCI situation</td>
</tr>
<tr>
<td>Extent (1-2-3-vessel disease)</td>
</tr>
<tr>
<td>Primary reperfusion</td>
</tr>
<tr>
<td>Time to primary reperfusion, h</td>
</tr>
<tr>
<td>Chronic total occlusion</td>
</tr>
<tr>
<td>Time to revascularization, days</td>
</tr>
<tr>
<td>Stent used (Cypher:Taxus)</td>
</tr>
<tr>
<td>Medication</td>
</tr>
<tr>
<td>Aspirin + clopidogrel</td>
</tr>
<tr>
<td>ACE inhibitor/AT-II receptor blocker</td>
</tr>
<tr>
<td>β-Blocker</td>
</tr>
<tr>
<td>Statin</td>
</tr>
</tbody>
</table>

There were no significant differences in any of the baseline parameters between the cell infusion and control group either with AMI or OMI. LAD indicates left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; ACE, angiotensin converting enzyme; AT, angiotensin.
in the cell infusion group (0.56±0.38 to 1.30±0.77 mg/dL; P<0.001) compared with the control group (0.55±0.33 to 0.50±0.40 mg/dL; P>0.05).

We infused 1.4±0.5×10⁶ collected leukocytes (volume: 7.8±3.2 mL) via over-the-wire balloon angioplasty catheter after PCI into the infarcted myocardium in patients from the cell infusion group. The composition of the mobilized and infused cells were: 9.3±10.2% CD34+, 15.1±15.9% KDR+, 2.2±7.4% AC133+, and 5.7±10.4% CD34+/KDR+. Notably, the infused cells in AMI patients contained more KDR+ cells (19.4±18.4% versus 9.7±10.3%; P<0.05) and marginally more CD34+/KDR+ cells (8.0±12.7% versus 2.0±2.6%; P=0.062) than those in OMI patients. There was no evidence for myocardial damage associated with cell therapy, as measured by CK-MB levels (P>0.05). There was no procedure-related serious adverse reactions during apheresis and cardiac catheterization. Also, there was no MACE during the hospitalization.

A total of 5 patients dropped out before 6-month follow-up; 1 patient in the AMI cell infusion group and 3 patients in the AMI control group moved to different hospitals, and 1 patient in the AMI cell infusion group was dropped because he could not visit the outpatient clinic because of severe degenerative arthritis. We monitored the general conditions of all of the patients by telephone, and none experienced serious adverse effects. There were 2 mortalities; 1 patient in the AMI control group because of reinfarction at 1 month and 1 patient in the OMI cell infusion group because of pancreatitis at 2 months after enrollment. In addition, 2 patients (1 each from the AMI control and OMI control group) experienced target vessel revascularization at 6-month follow-up.

### LV Systolic Function, Infarct Volume, and Remodeling by MRI

Baseline LVEF and LV volume were similar among study groups (Tables 2 and 3). AMI patients at 6 months showed significant improvement in LVEF compared with baseline in the cell infusion group (52.0±9.9% to 57.2±8.7%; P=0.01), and the change in LVEF was significantly greater in the cell infusion compared with the control group (+5.1±9.1% versus −0.2±8.6%; P=0.046; Figure 2A). LV end-systolic volume was significantly decreased in the AMI cell infusion group compared with the AMI control group (−5.4±17.0 mL versus +6.5±21.9 mL; P=0.04). Moreover, LE volume decreased significantly in the AMI cell infusion group compared with the AMI control group (−12.5±13.3 mL versus +0.8±14.3 mL; P<0.01). In OMI patients, however, there were no significant longitudinal changes of LVEF, LV volume, or LE volume in both the cell infusion and control group (Figure 2B).

### CFR

After stenting, baseline CFR measured in the culprit artery was similar between the cell infusion and control group in both AMI and OMI patients. At follow-up, there was a significant increase in CFR in both groups of AMI patients, whereas only the cell infusion group showed significant improvement in OMI patients (P<0.01; Tables 2 and 3).

### Angiographic Follow-Up

At 6 months, only 4 patients in the control group (2 each in AMI and OMI) showed angiographic binary restenosis. The late luminal loss was not significantly different between the cell infusion and control group, suggesting that G-CSF administration and stem cell infusion after DES implantation did not aggravate neointimal growth (Figure 3). QCA analysis of the distal nonstented segment also showed no significant difference in late luminal loss between the 2 groups.

### Discussion

The 6-month follow-up result of the MAGIC Cell-3-DES trial showed that intracoronary infusion of mobilized PBSCs with G-CSF improved LV systolic function and remodeling preferentially in patients with AMI, whereas its effect on LV
systolic function in those with OMI was insignificant. We also reconfirmed the safety and feasibility of intracoronary infusion of G-CSF–mobilized PBSCs in patients with AMI and OMI. Furthermore, restenosis after G-CSF therapy and cell infusion, previously suggested from the results of the original MAGIC Cell-1 trial,5 was not observed in this study using DES.

Influences of Stem Cell Therapy on LV Function, Remodeling, and Microcirculation
The LVEF increased by an absolute $5.1\pm 9.1\%$ at 6 months after intracoronary PBSC infusion in patients with AMI. However, in patients with OMI, we could not find any significant improvements in LV systolic function after cell therapy. The improvement observed in AMI was comparable with other studies,2,13 as well as our previous study.5 However, results in OMI patients were different from other studies that showed significant improvement of the LVEF with recanalization of chronic occlusion followed by PBSC14 or bone marrow cell15 infusion. The discrepancy between our results and others may be related to cell types and extent of hibernating myocardium. The study by Erbs et al14 used similar strategies to ours but showed quite different outcomes in change of LV systolic function, which might be related to differences in baseline characteristics of patients. In OMI patients of our study, CFR was improved, and infarct volume in MRI tended to decrease with cell infusion as Erbs' group did,14 which suggests that the disparity in the extent of hibernating myocardium in peri-infarcted segments may be related to the difference in recovery of contractility. In addition, duration of chronic total occlusion was shorter in patients from Erbs' study14 compared with our study. In subgroup analysis of our patients with chronic total occlusion, cell infusion did not improve LVEF after recanalization (n=6). Strauer et al15 injected bone marrow cells, which are
known to include more mesenchymal stem cells than PBSCs, and thus may have more potential for myocardial regeneration resulting in favorable influence on OMI.

It has still not been clarified what mechanism accounts for improvement of ventricular function after stem cell transplanation after MI. We can guess at least 2 possibilities: (1) direct differentiation of infused stem cell, and (2) paracrine effects by infused cell, which stimulate resident cardiac stem cells to proliferate and differentiate into cardiac or vascular cells. However, the relative contribution of 2 mechanisms could not be determined.

Angiogenesis has been regarded as a plausible mechanism for the prevention of remodeling and rescue of hibernating myocardium. In our study, cell infusion improved coronary microcirculation of infarct-related artery to a greater extent compared with the control group. In OMI, improvement of CFR was significantly greater in the cell infusion group than the control group, although it was insignificant in AMI. The disparity may come from mechanisms of improving microcirculation. In AMI, resolution of “microvascular stunning” because of microthrombi or tissue edema associated with acute infarction itself and revascularization is more important than cell therapy. However, in OMI, CFR mainly reflects the effect of cell therapy and revascularization. Thus, preferential improvement of CFR in the OMI cell infusion group suggests a beneficial effect of cell therapy on coronary perfusion.

In addition to angiogenesis, several potential mechanisms, such as myogenesis, rejuvenation by cell fusion, and paracrine effects, have been postulated to affect remodeling after stem cell therapy. In our study, improvement of LV systolic function and remodeling cannot be explained by the extent and presence of hibernating myocardium alone. This suggests that mechanisms other than angiogenesis may be involved. Infarct size measured as LE in MRI was reduced by cell infusion significantly in AMI, although marginally in OMI patients. This finding suggests that cell therapy may induce not only angiogenesis but also myogenesis, that is, angio-myogenesis, leading to reduction of infarct size.

G-CSF itself has several potential mechanisms that can favorably influence the outcome of stem cell therapy. Moreover, G-CSF showed enhanced endothelial progenitor cell colony-forming ability and increased chemokine receptor expression important for progenitor cell homing and engraftment.

**Different Outcomes of Stem Cell Therapy in AMI Versus OMI**

We evaluated PBSC distribution 4 hours after intracoronary coinfusion of 2-[18F]-fluoro-2-deoxy-D-glucose–labeled PBSCs in 16 patients and observed that 1.6 ± 0.7% of the labeled PBSCs were detected in the infarcted myocardium (data not shown), which is similar to another study with bone marrow cells. Despite a different outcome with cell infusion, integration of infused stem cell is not different between patients with AMI and with OMI, suggesting that the interaction of underlying milieu and integrated stem cells after entrapment during the first pass after infusion may play an important role in outcome. The finding that the cells mobilized in AMI patients contained more stem/progenitor cells might be one possible explanation for preferential improvement of LV systolic function in AMI patients. However, the characteristics of the cells mobilized by G-CSF might be more responsible for the difference. Because PBSCs contain mainly more endothelial progenitor cells than mesenchymal stem cells, angiogenesis would contribute more in our study. In OMI, mostly infarcted myocardium is replaced by scar, allowing little room for improvement by angiogenesis, explaining the minimal effect of stem cell therapy on contractile function in OMI. However, in AMI, acute ischemia upregulates various stimuli that induce differentiation and homing of stem cell, which may enhance the outcome of stem cell therapy. Our results suggest that stem cell therapy should be optimized according to the underlying condition of patients suffering from LV dysfunction.

**Safety and Feasibility of G-CSF Mobilized Stem Cell Infusion Combined With DES**

We previously observed higher rates of restenosis in patients treated with G-CSF. G-CSF treatment was associated with greater late luminal loss and higher restenosis, especially when injected before PCI. In our previous study, G-CSF–mediated neointimal growth was associated with mobilization of smooth muscle progenitor cells and inflammation induced by G-CSF. In this situation, DES was effective to prevent neointimal growth aggravated by G-CSF, and G-CSF in turn facilitated re-endothelialization of DES. We also reported the beneficial effect of stem cell mobilization to enhance re-endothelialization with vascular brachytherapy. Therefore, we expected that the combination of DES and G-CSF would be safe and effective. With this combination, neointimal growth was not worse in patients who received G-CSF than control or previous studies using DES without stem cells. Additionally, cell infusion mobilized by G-CSF also did not aggravate de novo atherosclerotic lesions.

During follow-up, we observed 3 cases of MACE only in the control group (1 fatal MI and 2 cases of target vessel revascularization). G-CSF–based stem cell therapy was safe and feasible in patients with MI.

**Conclusions**

Six-month follow-up result of the MAGIC Cell-3-DES Trial shows that intracoronary infusion of mobilized PBSCs by G-CSF significantly improves LV systolic function and reduces infarct size, compared with those receiving only PCI, in patients with AMI. Such efficacy of cell therapy is less definite in patients with OMI in spite of enhanced coronary perfusion, which needs further larger study. G-CSF administration with the exclusive use of DES in stem cell therapy is both feasible and safe and may eliminate any potential for restenosis suggested from previous studies.

**Sources of Funding**

This study was supported by a grant from Stem Cell Research Center, Republic of Korea (SC3150, to Dr Y-B Park).

**Disclosures**

None.
References


Kang et al G-CSF and Cell Infusion in Myocardial Infarction I-151


Downloaded from http://circ.ahajournals.org/ at CONS KESLI on June 17, 2014