Lack of Additional Benefit of Intracoronary Transplantation of Autologous Peripheral Blood Stem Cell in Patients With Acute Myocardial Infarction

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Background Recently the potential of myocardial repair by transplantation of autologous bone marrow stem cells has been suggested. Whether the additional intracoronary transplantation of autologous peripheral blood stem cells (PBSC), which were mobilized by granulocyte-colony-stimulating factor (G-CSF), could safely improve myocardial function in patients with acute myocardial infarction (AMI) was investigated.

Methods and Results Seventy-three patients with AMI who had successfully undergone percutaneous coronary intervention (PCI) were enrolled in the present prospective nonrandomized open-labeled study. Ten patients with elective PCI received G-CSF for 4 days followed by intracoronary PBSC transplantation. Thirty-two patients with primary PCI and 31 patients with recent AMI and elective PCI served as controls. The left ventricular (LV) function was evaluated using echocardiography and magnetic resonance imaging. G-CSF and intracoronary transplantation of PBSC did not incur any periprocedural myocardial damage. After 6 months, the LV ejection fraction was significantly improved in the cell therapy group. For 2 years of the follow-up period, there was no adverse clinical events, except one asymptomatic in-stent restenosis. However, comparable improvement of the LV ejection fraction was also identified in the primary PCI and elective PCI control groups.

Conclusions In the present study, additional intracoronary infusion of PBSC was safe and feasible for the patients with AMI who had undergone PCI, but did not lead to a significant improvement in LV function compared to standard reperfusion treatment. (*Circ J* 2007; 71: 486–494)

Key Words: G-CSF; Left ventricular function; Myocardial infarction; Stem cell

In the treatment of acute myocardial infarction (AMI), loss of myocardium and subsequent heart failure remain a major challenge despite major advances in the rapid reperfusion technologies.¹ On the basis of recent experimental studies that suggested the profound benefit of stem cell therapy for myocardial regeneration or healing, several clinical trials have explored the hypothesis that intracoronary infusion of autologous bone marrow stem cells may enhance the recovery of left ventricular (LV) function after AMI.^{2,3} However, the results are still not conclusive and also the risk of adverse cardiac events has been addressed.^{4–11}

To assess the safety and effectiveness of autologous adult stem cell transplantation in patients with AMI, we conducted an open-labeled phase II prospective clinical trial.

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Granulocyte colony stimulating factor was used for the mobilization of peripheral blood stem cells, because granulocyte-colony-stimulating factor (G-CSF) is a well-known peripheral blood stem cells (PBSC) mobilizer and the mobilization of PBSC is less invasive than direct bone marrow aspiration followed by cell processing.¹²

Methods

Patients

Between August 2003 and April 2004, we enrolled a series of 73 patients within 14 days of anterior ST-segment elevation AMI, who had received successful reperfusion treatment by percutaneous coronary intervention (PCI). We enrolled only patients with the proximal left anterior descending coronary artery (LAD) or mid LAD above first diagonal branch as the culprit vessel due to the following reasons. First, the location of the infarct on the site of vessel occlusion is known to affect the improvement of LV dysfunction,^{13–16} and clinical studies have shown that the LV ejection fraction is lower in anterior myocardial infarction (MI) than the inferior or lateral MI of the same infarct size.^{17,18} Second, the infarct location was similar to the experimental models in which the profound effect of cell therapy was shown.^{2,3,19} To avoid the dilution of any treatment effect, we excluded patients who presented within 2 h of symptom onset or patients with other small infarctions. Patients with persistent severe LV dysfunction (LV ejection fraction less than 25%), uncontrolled myocardial ischemia

Fig 1. The study profile. PCI, percutaneous coronary intervention; G-CSF, granulocyte-colony-stimulating factor; LV, left ventricular.

or ventricular arrhythmia, serious current infection or hematological disease and a life expectancy of less than 1 year were also excluded. We compared the results of cell therapy simultaneously with the primary PCI group, which is the current recommended treatment strategy and is proved to improve LV function, with the elective PCI group that had the same AMI-to-PCI time interval to the cell therapy group^{20–22} The institutional review board of the Samsung Medical Center approved the study protocol and written informed consent for each treatment was obtained from all participants.

Study Design

The overall trial profile is shown in Fig 1. Patients who had undergone PCI within 12h of the onset of symptoms comprised the primary PCI group (n=32). Cell therapy was planned for patients who undergone PCI beyond 12h of the onset of symptoms. After considering the experimental nature of cell therapy and the recommendations from institutional review board, patients who agreed to cell therapy were enrolled into the cell therapy group (n=10), and patients who refused additional cell therapy served as the delayed PCI group (n=31).

Procedures

In cell therapy group, PBSC were mobilized by daily subcutaneous injections of G-CSF 600 µg ($8.6 \pm 1.3 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, Dong-A pharmaceutical, Seoul, Korea) for 4 days. PBSC was obtained using COBE spectra apheresis system (COBE BCT Inc, Lakewood, CO, USA) at day 4 (5.9 ± 2.7 days after PCI), and followed by transradial cardiac catheterization immediately after apheresis. A total of 10 ml PBSC containing $2.03 \pm 0.69 \times 10^9$ ($1.13\text{--}3.25 \times 10^9$) mononuclear cells, which contains $3.12 \pm 2.19 \times 10^6$ ($0.9\text{--}8.3 \times 10^6$) CD34+ cells, was infused into the infarct-related coronary artery through a microinfusion catheter (Excelsior, Boston Scientific, MA, USA) using a 3–4 fractional manual infusion. To allow adherence and to maximize the potential transmigration

of the infused cells through coronary vascular endothelium, intravascular flow was stopped for 60s at the end of each infusion using a low-pressure inflation of intracoronary balloon occlusion system (Guardwire, Medtronic, MI, USA).

Characterization of Infused Cells

The PBSC apheresis product, which consisted of a heterogeneous cell population, was characterized by fluorescence-activated cell sorter analysis (FACSCalibur, Becton Dickinson, CA, USA) using directly conjugated antibodies against anti-human CD34, anti-CD14, anti-KDR, anti-VE cadherin and anti-vWF (BD Biosciences). PBSC included $0.15 \pm 0.07\%$ of CD34+ cells, $0.74 \pm 0.39\%$ of KDR+ cells, $0.09 \pm 0.09\%$ of VE cadherin+ cells and $1.52 \pm 0.93\%$ of vWF+ cells.^{3,23}

Assessment of LV Function

Two-dimensional echocardiography was performed 4 days (range 3–5) after PCI and at 6 months' follow-up for all participants (Sequoia C512, Acuson, Siemens Medical Systems, PA, USA). LV ejection fraction was calculated using the biplane modified Simpson's method. The wall motion score index was calculated from the average sum of wall motion scores. Cardiac magnetic resonance imaging (MRI, 1.5-T system; GE Signa CVi, General Electric Medical Systems, Milwaukee, WI, USA) was performed in the cell therapy group and the primary PCI group at day 4 (range 3–5) and after 6 months. All images were acquired using a cardiac phase-array coil during breath-holds and were electrocardiographically-triggered. Cine images of the entire left ventricle were acquired in contiguous short-axis views using Fast Imaging Employing Steady-state Acquisition method with a field-of-view (FOV) of 250 mm and 6 mm slice thickness. The LV ejection fraction and regional wall motion was analyzed using the MASS 4.0.1 software (Medical Imaging Systems, Leiden, The Netherlands). First-pass perfusion imaging was obtained after intravenous infusion of gadolinium-diethylenetriaminepentaac-

Table 1 Demographic, Clinical, and Procedural Characteristics of the Study Population

	Cell therapy (n=10)	Primary PCI (n=32)	Delayed PCI (n=31)
Age	50.5±10.6*	55.1±11.2	64.4±9.2
Men, n (%)	10 (100)	29 (91)	24 (77)
Diabetes, n (%)	4 (40)	9 (28)	12 (39)
Hypertension, n (%)	3 (30)	15 (47)	15 (48)
Hyperlipidemia, n (%)	2 (20)	5 (16)	4 (13)
Smoking, n (%)	5 (50)	17 (53)	10 (32)
Previous revascularization, n (%)	0 (0)	1 (3)	2 (6)
Previous stroke, n (%)	0 (0)	1 (3)	1 (3)
Time to revascularization, mean/median, days	8.3±8.2/7	0.3±0.3/0	8.4±7.1/5
Maximal CK-MB, U/L	174±266	241±106	45±60*
Medications on discharge			
Aspirin and clopidogrel, %	100	100	100
-blocker, %	100	97	97
ACE inhibitor, %	100	100	100
Statin, %	100	100	100
Culprit vessel			
Proximal LAD, n (%)	7 (70)	22 (69)	15 (48)
Mid LAD, n (%)	3 (30)	10 (31)	16 (52)
Primary Device			
Drug-eluting stent, n (%)	6 (60)	8 (25)	11 (36)
Bare metal stent, n (%)	3 (30)	23 (72)	19 (61)
Balloon only, n (%)	1 (10)	1 (3)	1 (3)
Device diameter (mm)	3.50±0.33*	3.27±0.38	3.11±0.38*
Device length (mm)	20.70±4.79	21.81±6.15	22.13±7.54
TIMI grade (baseline)	1.3±1.3	0.53±1.0	2.3±1.1*
TIMI grade (after PCI)	3.0±0.0	3.0±0.0	3.0±0.0

PCI, percutaneous coronary intervention; CK-MB, creatine kinase myocardial band; ACE, angiotensin-converting enzyme; LAD, left anterior descending artery; TIMI, Thrombolysis in Myocardial Infarction.

The device diameter and device length denote the size of stent used.

Data are mean±SD when appropriate.

etate (Magnevist, Schering, Berlin, Germany; 0.15 mmol/kg body weight in total amount at 3 ml/s). The slice thickness was 6 mm with a FOV of 40×40 cm and image matrix of 128×128 and images of 6 or 7 locations. Every 2 heartbeats were obtained for 40 phases using a fast gradient echo train sequence (repetition time (TR)/echo time (TE)=7.3 ms/1.8 ms). Late enhancement imaging was performed using an inversion-recovery pulse prepared fast gradient echo recalled technique (TR/TE/inversion time=5.5 ms/1.4 ms/200 ms; slice thickness, 6 mm with 10 mm interslice gap; FOV, 40×40 cm; image matrix, 256×160).

Evaluation of Coronary Artery Restenosis and Coronary Hemodynamics

Follow-up angiography was performed at 6 months after enrollment in all cell therapy group patients, 75% of the primary PCI group (n=24) and 55% of the delayed PCI group (n=17). In these patients, quantitative coronary angiography (QCA) was done as the baseline, immediately after PCI and at 6 months' follow-up with Philips QCA software (Pie medical imaging, Maastricht, Netherlands). Identical unforeshortened coronary projections were used and analyzed. Binary restenosis was defined as more than 50% loss of luminal diameter within the stent±5 mm.

In cell therapy group, we evaluated the effect of cell infusion on myocardial microcirculation through measurement of the coronary flow reserve of the infarct-related artery, immediately before and after cell infusion and at 6 months' follow-up. A coronary Doppler wire was positioned just distal to the implanted stent in the culprit lesion and an intracoronary bolus injection of 18 µg adenosine was used to induce hyperemia.⁴

Parameters of Safety and Feasibility

All patients underwent regular follow-up visits for assessment of their clinical status, any adverse reactions and their medication at 1 month and every 3 months thereafter. Patients were treated with aspirin, clopidogrel, -blockers, angiotensin-converting enzyme (ACE) inhibitor and statin, unless these agents were contraindicated. Clinical, treatment and safety laboratory data were prospectively collected by research nurses. The safety of the cell therapy was assessed on the basis of the development of major adverse cardiac events and their clinical status, including G-CSF related pain, biochemical tests and arrhythmia.

Statistical Analysis

Continuous variables are presented as the mean±SD or median. Categorical variables are compared using the chi-square test or Fisher's exact test. Statistical comparisons between the initial and follow-up data were performed in a nonparametric manner using the Wilcoxon signed rank test. One-way ANOVA was performed to evaluate differences between groups. A trend in survival scores across the groups was calculated using a log-rank test for trends. All tests were two-tailed and p-values of less than 0.05 were considered to be statistically significant. We used SPSS (version 11.5, SPSS Inc) for statistical analyses.

Results

Study Population

The demographic and clinical characteristics of the study population are summarized in Table 1. Aspirin, clopidogrel, -blockers, ACE inhibitors and statin were initiated and continued in all patients during study period, except 2

Table 2 Echocardiographic Evaluation of LV Function

	Cell therapy	Primary PCI	Delayed PCI
<i>LVEF (%)</i>			
Basal	40.1±6.5	42.6±6.3	42.1±8.0
6 month	49.4±13.4	48.6±7.1	48.5±12.3
Change	9.3±12.5	6.0±7.7	6.5±8.9
<i>p value</i>	0.047*	0.001*	0.002*
<i>WMSI</i>			
Basal	1.87±0.37	1.82±0.24	1.80±0.36
6 month	1.60±0.46	1.56±0.34	1.61±0.45
Change	-0.27±0.37	-0.26±0.39	-0.21±0.38
<i>p value</i>	0.038*	0.001*	0.001*

LV, left ventricular; LVEF, LV ejection fraction; WMSI, wall motion score index. Other abbreviation see in Table 1.

Data are mean±SD.

* $p < 0.05$ between initial and 6-months follow up (Wilcoxon Signed Ranks test).

Fig 2. Improvement of left ventricular (LV) function evaluated by echocardiography. LV ejection fraction (LVEF), measured initially and at 6-months (6mo) follow-up, increased significantly in all groups (A–C). LV wall motion analyzed by wall motion score index was also improved significantly in all groups (D–F). * $p < 0.05$ between initial and 6-months follow-up (Wilcoxon Signed Ranks test). PCI, percutaneous coronary intervention; WMSI, wall motion score index.

patients who stopped taking β -blockers due to symptomatic sinus bradycardia. The cell therapy group was younger compared to the other groups. The time to revascularization was shorter in the primary PCI group. Maximal creatine kinase (CK)-MB was significantly lower in the delayed PCI group. Baseline Thrombolysis in Myocardial Infarction grade was significantly higher in the delayed PCI group.

Safety Issues

The peripheral white blood cell count reached up to $3.51 \pm 0.90 \times 10^4 \text{ mm}^{-3}$ after G-CSF treatment in cell therapy group, which was significantly higher than in the primary PCI group ($1.23 \pm 0.34 \times 10^4$) or delayed PCI group ($0.96 \pm 0.39 \times 10^4$) ($p = 0.001$). Intracoronary cell infusion did not induce a significant elevation of CK-MB ($2.3 \pm 3.2 \text{ ng/ml}$ after 24h), which is less than the cut-off value (5 ng/ml). Furthermore, cell infusion did not affect coronary microcir-

culation, which was assessed using the coronary flow reserve (before vs after infusion, 1.90 ± 0.5 vs 1.82 ± 0.49 , $p = \text{NS}$).

G-CSF treatment was well tolerated in all patients. There were no symptoms or signs of myocardial ischemia or arrhythmia in the periprocedural period. There was also no other serious adverse actions or clinical events such as G-CSF-related pain. In-hospital telemetric monitoring and 24h Holter monitoring at 6 months revealed no ventricular arrhythmia in the cell therapy group.

Improvement of LV Function

At 6 months after PCI, the global LV function evaluated using echocardiography improved significantly in all groups. The relative improvement in the LV ejection fraction was $9.3 \pm 12.5\%$ for cell therapy group, $6.0 \pm 7.7\%$ for primary PCI group and $6.5 \pm 8.9\%$ for delayed PCI group. The regional wall motion evaluated using the wall motion

Table 3 Evaluation of Global and Regional LV Function by MRI

	Cell therapy	Primary PCI
LVEF (%)		
Basal	37.9±8.9	41.8±7.0
6 month	45.2±11.0	47.8±9.8
Change	7.3±7.8	6.0±9.0
p value	0.033*	0.019*
LVEDV index (ml/m²)		
Basal	89.5±28.4	83.2±22.4
6 month	91.2±32	75.2±19.1
Change	1.8±22.9	-7.9±19.2
p value	0.889	0.121
LVESV index (ml/m²)		
Basal	57.5±25.6	50.1±17.2
6 month	52.9±29.0	44.4±16.8
Change	-4.5±21.2	-5.7±9.4
p value	0.441	0.044*
LVM index (g/m²)		
Basal	58.8±8.8	60.3±15.0
6 month	58.7±13.9	54.8±14.2
Change	-0.2±12.1	-5.2±11.7
p value	0.515	0.109
SWM, all (mm)		
Basal	5.0±1.9	6.1±1.4
6 month	6.2±1.9	6.7±1.6
Change	1.4±1.4	0.6±1.4
p value	0.066	0.121
SWM, infarct area (mm)		
Basal	3.8±1.6	4.5±1.0
6 month	5.3±1.7	5.9±1.5
Change	1.5±1.4	1.5±1.4
p value	0.015*	0.002*
SWM, non-infarct area (mm)		
Basal	6.0±2.0	7.2±2.3
6 month	7.1±2.3	7.8±2.0
Change	1.1±1.9	0.6±1.7
p value	0.110	0.179
Late contrast hyperenhancement (g)		
Basal	21.5±9.6	22.8±11.4
6 month	17.7±14.9	15.1±7.8
Change	-3.8±11.7	-7.7±12.2
p value	0.345	0.071

MRI, magnetic resonance imaging; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVM, left ventricular mass; SWM, systolic wall motion. Other abbreviations see in Tables 1,2.

Data are mean ± SD when appropriate.

*p<0.05 between initial and 6-months follow up (Wilcoxon Signed Ranks test).

score index also improved significantly in all groups. The degree of improvement of the LV ejection fraction and the wall motion score index was not significantly different among groups (Table 2, Fig 2).

Paired MRI analysis revealed that the global LV function also improved significantly in both the cell therapy and primary PCI group. The relative improvement in the LV ejec-

tion fraction was 7.3±7.8% for the cell therapy group and 6.0±9.0% for the primary PCI group. Analysis of the regional LV systolic wall motion revealed that the improvement of LV function was primarily derived from the improvement of the wall motion of the infarcted myocardium. The volume of late hyperenhancement, which is closely related to the irreversibly damaged myocardium, had a tendency to decline after 6 months but this was statistically not significant in both groups (Table 3, Fig 3)?

The coronary flow reserve of the cell therapy group improved from the baseline value of 1.82±0.49 to 2.84±0.79 at 6 months (p=0.006), which was similar to previous reports^{4,21,24}

Clinical Course

With respect to clinical outcomes at 6 months, there were no deaths, MIs or hospital admissions in the cell therapy or primary PCI groups. In the delayed PCI group, there were 2 cardiac deaths. Also 4 patients of the delayed PCI group required hospital admission for the treatment of heart failure. Angiographic follow-up was performed in all cases of the cell therapy group at 6 months. We identified 1 angiographic in-stent restenosis (diameter stenosis=50%) at the site of sirolimus-coated stent implantation, but PCI was not performed because the patient was asymptomatic. In the primary PCI or delayed PCI groups, the 6-month angiographic follow-up was performed in 24 patients (67%) and 16 patients (52%), respectively. In-stent restenosis was identified in 2 patients of primary PCI group and 5 patients of the delayed PCI group, and PCI was repeated in 1 and 3 patients, respectively. In these groups, all restenosis was occurred at the site of the bare metal stent implantation.

We followed the patients for 787±99 days. There were no additional deaths, MIs, revascularizations or hospital admissions in the cell therapy group. In the primary PCI group, 1 patient suffered sudden cardiac death and 2 patients have undergone elective PCI for progressed non-infarct-related lesions. In the delayed PCI group, there were 2 additional cardiac deaths and 1 additional rehospitalization due to heart failure. A survival analysis revealed that the combined risk of death, MI, revascularization and rehospitalization due to heart failure was the only significant trend across groups both at 6 months (cell therapy group: 0%; primary PCI group: 3.2±3.1%; delayed PCI group: 23.3±7.7%; p=0.027, log-rank test for trend) and at 2 years (0%; 6.8±4.7%; 35.8±9.4%; p=0.022) (Table 4).

Discussion

The findings of the present study indicate that intracoronary transplantation of G-CSF mobilized PBSC may be safe, which is consistent with previous studies, but it may

Fig 3. Improvement of left ventricular (LV) function evaluated by magnetic resonance imaging (MRI). LV ejection fraction (LVEF), measured initially and at 6-months follow-up, increased significantly in both the cell therapy and primary percutaneous coronary intervention (PCI) groups (A, B). Descriptive data are shown in Table 3. *p<0.05 between initial and 6-months follow-up (Wilcoxon Signed Ranks test).

Table 4 Clinical Follow up Results

	Cell therapy (n=10)	Primary PCI (n=32)	Delayed PCI (n=31)
<i>6 months follow up</i>			
Non-fatal MI, n (%)	0 (0)	0 (0)	0 (0)
Target lesion revascularization, n (%)	0 (0)	1 (3)	3 (10)
Death, n (%)	0 (0)	0 (0)	2 (6)
Hospitalization due to heart failure, n (%)	0 (0)	0 (0)	4 (13)
MACE, n (%)	0 (0)	1 (3)	3 (10)
MACE + admission due to heart failure, n (%)	0 (0)	1 (3)	7 (23)
<i>2 years follow up</i>			
Non-fatal MI, n (%)	0 (0)	0 (0)	0 (0)
Target lesion revascularization, n (%)	0 (0)	1 (3)	3 (10)
Death, n (%)	0 (0)	1 (3)	4 (13)
Hospitalization due to heart failure, n (%)	0 (0)	0 (0)	5 (16)
MACE, n (%)	0 (0)	2 (6)	7 (23)
MACE + admission due to heart failure, n (%)	0 (0)	2 (6)	12 (39)

MI, myocardial infarction; MACE, major adverse cardiac event, which is defined by death, MI, or target lesion revascularization.

Other abbreviation see in Table 1.

The combined risk of death, MI, revascularization, and rehospitalization due to heart failure is the only significant trend across groups both at 6 months and at 2 years ($p=0.022$).

* $p<0.05$ (log-rank test for trend).

not enhance the recovery of global LV function beyond that obtained by standard reperfusion therapy.^{7,25} Cell therapy did not contribute to an additional increase in LV ejection fraction when evaluated using echocardiography and MRI. Also the changes in LV volumes over 6 months were not different significantly between cell therapy group and the other groups, which suggests a minimal effect on the LV remodeling.^{9,25}

Benefit of Post-PCI Cell Therapy in AMI Patients

The present study raises the issue of whether the bone marrow cells can enhance the improvement of LV function after AMI. The potential benefits of stem-cell transplantation were first shown in experimental animal MI models with large anterior infarctions.^{2,3} However, the profound beneficial effect has not been reproducibly observed in clinical studies. The range of the improvement of LV ejection fraction varied from 0 to 8.6%^{4–10,25,26} In addition, there are still controversies about the myocardial regenerative capacity of adult stem cells.^{27–31}

It has been well known that early reperfusion after AMI reduces the infarct size and preserves LV function. Recent studies using meticulous echocardiography or MRI analysis showed a 3 to 7 percentage point improvement in ejection fraction and a reduction of infarct size after primary PCI.^{20,21,32–34} In addition, recent randomized controlled clinical trials showed that LV function can also improve significantly after a late re-opening of the infarct-related artery.^{32,35}

In the present study, we excluded patients with a high risk of developing congestive heart failure who might have enjoyed maximal benefits of stem cell therapy. In contrast, if ventricular function is too well preserved, functional improvement from stem cell therapy may be hardly expected. We were also concerned about the location of infarct and the site of vessel occlusion, which is known to affect the improvement of LV function and has been strictly controlled in successful animal models.^{13–18} To overcome the potential bias described above, we enrolled only patients with proximal to mid LAD infarction. All our patients had a similar vessel occlusion site and large infarction size, which is most alike to the experimental models in which

the profound effects of cell therapy were shown.

Although the clinical endpoint defined by the improvement of LV systolic function and the time to revascularization was similar between the cell therapy and delayed PCI groups, the cell therapy group has a shown comparable improvement of LV function despite of a higher maximal CK-MB compared to delayed PCI group (174 ± 266 versus 45 ± 60 U/L). Furthermore, although it was not included as clinical endpoint at the stage of study design, there was a significant trend toward better clinical consequences in cell therapy group. A survival analysis showed that the combined risk of death, MI, revascularization and rehospitalization due to heart failure was better in cell therapy group (0%) compared to primary PCI group (3.2%) and delayed PCI group (23.3%) after 1 year. So the potential benefits of cell therapy on myocardial function should be considered. First, the replacement of lost myocardial cells through the myocardial differentiation of infused cells, which was initially proposed, could be taken into account. However, subsequent studies failed to support these initial observations.^{30,36} And it might have been confused with cellular fusion.^{37,38} So the likelihood of the differentiation of stem cells into functional cardiomyocytes would hardly exist. Second, the augmentation of resident cardiac stem cell proliferation by infused stem cells could be considered. Direct myocardial regeneration was identified in animals,^{39–42} and animal cardiac stem cells were suggested.^{43–45} However, the existence of human cardiac stem cells has not been clearly proven yet.⁴⁶ Third, the locally transplanted cells could act in a paracrine fashion to improve myocardial function through the release of growth factors or other paracrine mediators. Human adult stem cells mobilized by G-CSF are rich in bioactive cytokines, including many growth factors. These mediators may prevent apoptosis of ischemic myocardial cells or limit injury by promoting angiogenesis rather than tissue regeneration.^{47,48} Pre-clinical studies have shown improvement in angiogenesis or the salvage of ischemic tissue by direct implantation of human adult stem cells on the ischemic skeletal or heart muscle.^{23,49–51} Fourth, the effect of cytokine used for the cell mobilization should be considered. G-CSF has been known to inhibit the apoptotic death of cardiomyocytes, increase arteriogenesis

and augment intercellular connection. Therefore, G-CSF has been reported to lead to the promotion of the salvage of the injured myocardium, prevention of ventricular remodeling and reduction of inducible arrhythmia.^{52–54} These hypotheses could be applied to the results of the present study, which were a comparative improvement of LV function in cell therapy group compared to the delayed PCI group despite a higher maximal CK-MB in the cell therapy group.

The cell processing method or cell dose might be an important issue in cell therapy. We used continuous G-CSF injection and peripheral blood stem cell apheresis followed by immediate intracoronary cell infusion, which has been tried in previous studies very successfully.^{4–6,8,9,24} The dose of cells used in the present study was $2.03 \pm 0.69 \times 10^9$ of total mononuclear cells and $3.12 \pm 2.19 \times 10^6$ of CD34+ cells, which was comparable to or higher than previous trials and was only less than the BOOST trial ($2.46 \pm 0.94 \times 10^9$ total mononuclear cell, $9.5 \pm 2.19 \times 10^6$ CD34+ cell), which showed no impact on intracoronary cell infusion on mid-term LV function.⁹ In a MI involving 30% of the left ventricle, the number of cardiomyocytes is known to be reduced by more than 1×10^9 . Only 1 to 2% of mononuclear cells in bone marrow are CD34+ progenitor cells, and true functional stem cells are even scarcer.⁵⁵ Furthermore, it has been shown that most of infused human mononuclear cells were simply washed out and only 1.5% of infused cells could accumulate in myocardial tissue,^{56,57} and a large proportion die after a few days.⁵⁸ Moreover, the most optimistic calculations from animal data indicate that the maximum amount of new myocardium generated during cell therapy would be 1 to 5 g, from which the functional contribution to the human adult heart could be hardly measured.^{59,60} The results of the present study suggest that the efficacy of post-PCI cell therapy is hardly conclusive, even in AMI patients most similar to the experimental AMI models with successful results.

In the present study, one patient in cell therapy group has shown to lack an improvement of LV function even with revascularization followed by stem cell therapy. It has been known that AMI patients treated with primary PCI had 3 to 7 percentage point improvement in ejection fraction when compared to the control groups.^{20,21,32–34} However, in 25% of treated patients, LV function deteriorates even with successful primary PCI.⁶¹ Our case described above may be a good example showing the limitations of cell therapy in the case of impaired improvement of LV function after primary PCI.

Safety Issues

There have been some concerns about the safety of G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) treatment for stimulating PBSC, because they may act as double-edged swords.⁶² Both G-CSF and GM-CSF have potent proinflammatory and procoagulant effects, and a number of cases of AMI and stroke have been reported.^{12,63} Recently 2 trials reported an apparent increase in acute coronary syndrome after the use of G-CSF or GM-CSF.^{64,65} One trial, which used G-CSF before PCI, reported a marked increase of in-stent restenosis.²⁴

In the present study, G-CSF treatment was started at 6 days after PCI. The dose was $8.6 \pm 1.3 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 4 days, which conforms to the current recommendation to not exceed $8.8 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$.¹² We did not identify any peri-procedural adverse events after G-CSF followed by intracoronary cell infusion. Target vessel revascularization as a

result of restenosis and late lumen loss in the G-CSF treated cell therapy group was also scarce for the 2 years of follow-up, which was shown in only 1 clinically insignificant in-stent restenosis. Our data suggest that if G-CSF were used within the recommended dose and with a sufficient time interval from PCI, G-CSF might not enhance the restenosis process or adverse cardiac events.^{24,64,65} However, further examination of the safety of G-CSF treatment in AMI patients should be investigated with more patients and longer follow-up periods.

There are several limitations in the present study. The study design, which was decided by investigators and institutional review board, was open-labeled, which is weak relative to a placebo-controlled blinded study. Also, the clinical characteristics of 3 study groups were not identical. The number of study participants was relatively small and underpowered for many endpoints.

In summary, the present study provided further confirmation of the feasibility and safety of additional intracoronary infusion of autologous peripheral blood stem cell in AMI patients after successful PCI. However, there was no additional treatment effect on the recovery of LV function compared with the substantial recovery found in placebo group, suggesting specific benefits beyond those offered by mechanical revascularization might be limited. Based on the present study, larger adequately-powered and well-controlled clinical trials would be required to confirm the impact of post-MI cell therapy on the recovery of LV function and mid-term survival.

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