

Safety and Feasibility of Combined Granulocyte Colony Stimulating Factor and Erythropoietin Based-Stem Cell Therapy Using Intracoronary Infusion of Peripheral Blood Stem Cells in Patients with Recent Anterior Myocardial Infarction: One-Year Follow-up of A Phase 1 Study

Teguh Santoso*, Cosphiadi Irawan*, Idrus Alwi*, Auda Aziz**, Agus Kosasih**, Sri Inggriani***, Ardian Saputra***, Merry Wintery***, Linda Lison***

*Department of Internal Medicine, University of Indonesia, Faculty of Medicine - Dr. Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta, Indonesia. ** Dharmais Cancer Hospital, Jakarta, Indonesia.

*** Medistra Hospital, Jakarta, Indonesia.

Correspondence mail to: tsantoso@cbn.net.id

ABSTRACT

Aim: to assess the safety and feasibility of combined granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO) based intracoronary peripheral blood stem cells (PBSCs) therapy in patients with recent myocardial infarction (RMI) who had successful reperfusion therapy with drug-eluting stent.

Methods: a total of 18 patients diagnosed with anterior ST-segment elevation AMI who had successful percutaneous coronary intervention (PCI) with drug-eluting stent implantation within 15 days after onset of symptom were enrolled. PBSCs were harvested and injected into the infarct-related artery after 5 consecutive days of G-CSF administration. Recombinant human erythropoietin was administered at the time of intracoronary PBSCs injection.

Results: there were no procedural and periprocedural complications, such as ventricular arrhythmia, visible thrombus formation, distal embolization, injury of the coronary artery associated with the cell infusion catheterization procedure or elevation of CK-MB of more than two-times. After PBSCs injection, all patients had grade III myocardial blush grade. At follow-up of 21.1 ± 5.5 months (range 12 to 30 months) there was no death, no re-infarction, no target lesion revascularization nor re-hospitalization for heart failure. Paired cardiac MRI demonstrated no change in left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV) at 3 months, but they increased significantly at one year. Despite this, left ventricular ejection fraction (LVEF), wall motion score index (WMSI) and perfusion score index (PSI) improved at 3 months and remained stable at one year. The percentage of late gadolinium enhancement to LV mass

(%LGE) were continuously improved until one year. There was no correlation between the level of CD34+, CD 45+, other cell subtypes as well as total number of PBSCs injected to the changes of LVEDV, LVESV, LVEF, WMSI, PSI, and %LGE ($p > 0.05$).

Conclusion: combined G-CSF and EPO based-intracoronary infusion of PBSCs in patients RAMI is safe and feasible.

Key words: stem cell, granulocyte colony stimulating factor, recent acute myocardial infarction.

INTRODUCTION

Current practice guidelines emphasize the importance of achieving reperfusion of the infarct-related artery (IRA) as early as possible.¹⁻³ However, primary percutaneous coronary intervention (PCI) is performed at less than 25% of acute care hospitals in the United States.^{4,5} Many patients with myocardial infarction with ST-segment elevation present to hospitals that do not have the capability of performing PCI and, therefore, cannot undergo PCI within the timelines recommended in the guidelines.¹⁻³ Instead, they receive thrombolytics as the initial reperfusion therapy. A report from the National Registry of Myocardial Infarction showed that 27.6% of the AMI patients received thrombolytic therapy in 2006.⁶ However, angiographic studies have shown that coronary reperfusion does not occur in 20% to 45% of patients receiving thrombolytic therapy.⁷ In addition,

5% to 30% of patients may experience early or late reocclusion.^{8,9} Furthermore even if timely executed, rapid reperfusion of IRA, either with thrombolytic therapy or PCI with stent implantation still results in an increasing proportion of patients with AMI surviving with significant LV dysfunction.

It is in this setting of desperation to treat a growing patient population at risk for heart failure that cardiovascular medicine has grabbed hold of the potential of stem cell therapy for myocardial tissue regeneration. Recent meta-analysis demonstrated safety and favorable effects of stem cell transplantation in patients with AMI.¹⁰⁻¹² In all reported studies, the time window from onset of AMI to stem cell therapy was usually within 24 hours after onset. A recent meta-analysis showed that PCI of the IRA performed late (12 h to 60 days) after AMI is still associated with significant improvements in cardiac function and survival.¹³ Therefore, it would be interesting to study the safety and feasibility of intracoronary stem cell therapy in late comers with large anterior wall infarction who presented days after their initial insult when significant remodeling process has taken place.

G-CSF-based stem cell therapy has been proposed as a practical and non-invasive alternative to stem cell therapy using bone marrow stem cells. G-CSF might be considered mostly as a mobilizer to enrich PBSCs. Despite the potential adverse effects of increasing vascular events, short term use of G-CSF in patients with AMI seems to be safe. In patients with AMI, intracoronary infusion of PBSCs improved cardiac function, whereas the administration of G-CSF alone did not.¹⁴⁻¹⁷

EPO treatment leads to significantly improved cardiac function following AMI. This protection is associated with mitigation of myocyte apoptosis, translating into more viable myocardium and less ventricular dysfunction. Importantly, cardioprotective effects of EPO are seen without an increase in hematocrit (eliminating oxygen delivery as an etiologic factor in myocyte survival and function), demonstrating that EPO can directly protect the ischemic and infarcted heart.¹⁸⁻²³ Whether the addition of EPO would enhance the effect of G-CSF-based stem cell therapy is unknown.

This study is conducted to evaluate the safety and feasibility of combined G-CSF and EPO based stem cell therapy using intracoronary infusion of PBSCs in patients who present days after harboring large anterior myocardial infarction and had successful reperfusion therapy with drug-eluting stent.

METHODS

We enrolled 18 patients diagnosed with ST-segment elevation anterior wall infarction who had successful PCI with drug-eluting stent implantation and were referred late (more than 2 hours) to our hospital but still within 15 days after onset of symptom. None of the patients received fibrinolytic therapy. PCI was performed regardless of the patients' hemodynamic condition. Other inclusion criteria were as follows: age 20 years or over and subject's willingness to comply with specific follow-up evaluations. All patients had complete revascularization even in non-infarct related territory. Exclusion criteria were: 1). hemodynamic instability during the procedure or any condition that may put the patient at undue risk such as pulmonary edema and cardiogenic shock, 2). previous or current severe co-existing diseases, such as cancer, hematological disorders (Hb <10 g/dL, WBC <4 or >11x10⁹/L, or platelets <100x10⁹/L), renal failure (creatinine level >2.5 mg/dL, or creatinine clearance <30 cc/min), hepatic dysfunction, serious infection or any co-morbidities that may impact patients' survival), 3). valvular heart disease and prosthetic valves, 4). hypertrophic or restrictive cardiomyopathy, 5). women of child bearing potential, and 6). lack of informed consent.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the University of Indonesia Medical School Ethical Committee. All patients gave written informed consent.

Study Protocol

One-to-three days after PCI, a daily dose of 10 µg/kg/day of G-CSF (Lenograstim [GranocyteTM], Aventis-Sanofi) was administered subcutaneously for 5 consecutive days. CD34+ and CD 45+ cells were quantified at days 1 (baseline), 3, and 5 after injection and subsequently the peripheral blood stem cells (PBSCs) were harvested. A total of 150 ml of PBSCs was processed from the brachial vein using COBE Spectra and placed into a peripheral blood (PB) unfiltered collection bag (Baxter Health Care Corp, IL), which was discarded after PB infusion. CD34+ and CD 45+ cells enumeration was performed using FACSCalibur (Becton Dickinson).

To ensure sterility of the harvesting procedure, the white cell concentrate was cultured for bacteria and fungi. Three milliliters of the cell concentrate was injected into BacT Alert PF Pediatric tube (Biomerieux Inc, Durham, NC, cat No. 259794) and the incubated in the BacT Alert 120 system (Organon Teknika, Durham, NC) for 7 days or until the culture become positive. The

cell concentrate was also cultured on Sabouraud agar medium (Oxoid, cat No. CM 0041) for fungi.

Patients were heparinized during stem cell injection. Cells were injected with the use of a stop flow technique through an over the wire balloon catheter positioned within the segment containing of the stent. The balloon was inflated with low pressure to completely block the blood flow for 3 min, during which time 5-6 ml PBSC suspension was injected. This was interrupted by 3 min of reflow by deflating the balloon to minimize the likelihood of extensive ischemia. After completion of intracoronary cell transplantation, coronary angiography was repeated to ascertain vessel patency and absence of capillary plugging by measuring the myocardial blush grade. Approximately 15-25 x 10⁶ PBSCs were injected during each procedure. All (except one) patients received subcutaneous 4000 IU of recombinant human erythropoietin (EprexTM) and glycoprotein IIb/IIIa inhibitors (IntergrellinTM) intravenous bolus and infusion at the time of intracoronary PBSCs injection. Patients were discharged the day after. Unless there is contraindication, all patients also received optimal standard therapy including aspirin, clopidogrel, nitrates, β -blockers, ACE-inhibitor/angiotensin-receptor blockers and statins.

The primary end point of the study was combined endpoints of death, myocardial infarction, stroke, or re-hospitalization for worsening heart failure. Secondary end-points were individual end-point of the combined end-points, angiographic restenosis, target vessel revascularization, the absolute change in regional (measured as WMSI) and global left ventricular function (measured as LVEF), perfusion (measured as PSI) and viability of infarct related area (measured as percentage of infarcted mass compared to total LV mass [%LGE]) as assessed with MRI 3 and 12 months after cell infusion.

Patient Assessment and Follow Up

All patients underwent baseline cardiac MRI after PCI. Clinical evaluations (assessments of functional status and adverse events) and biochemical analysis of blood samples were subsequently performed at one month and 3, and 6 months after myocardial infarction. Six months after myocardial infarction, coronary angiography were repeated. MRI was repeated at 3 and 12 months after index procedure

MRI scan was performed with 1.5 T Signa HDx (GE Medical Systems). Patient was placed in a supine position with an eight channels phase-array coil placed over thorax for signal reception. FIESTA Cine-MRI

was performed from base to apex of the heart in the four-chamber plane perpendicular to interventricular septum. Besides short axis imaging, four, three and two chamber imaging were also obtained. For LV segmentation, the standard 17 segment frame of reference was used. For quantitative analysis multiple parallel short axis slices covering the heart from the base-to- apex was obtained using a ECG-triggered breath-hold cine. The acquisition parameter was steady state acquisition TR 3.5 sec, TE 1, 6 sec, NEX 1, band width 125 KHz, no flip angle, matrix 224x224, FOV 400x400, slice thickness 8 mm, slice space 4-6 mm depending on the LV size, examined in the short axis, long axis and four-chamber views. Quantitative analysis was performed separately using standardized software by two observers. Segmental wall motion was assessed using semi-quantitative scoring: 0 was scored as normal, 1 as hypokinetic, 2 as akinetic, 3 as dyskinetic. The WMSI was equal to sum of scores divided by sum of segments. After assessment of resting cardiac function, LV myocardial Gadolinium as first-pass stress perfusion imaging was performed using adenosine as vasodilator (140ug/kg/min, via the contralateral antecubital venous access, in two minutes infusion of Gadovist (GadolbatrolTM Schering) 0.05 mmol/kg BW, rate of 5ml/sec followed by flush injection of 30 ml saline. MR sequence parameter is TR 3,0 m sec, TE 1.3 msec, no flip angle, matrix 128X 128, FOV 400X400, NEX 1, bandwidth 83.33 kHz, slice thickness 12 mm, slice space 6 mm.

Interpretation of myocardial perfusion was performed using visual qualitative analysis ('eye balling') by two independent observers. For semi-quantification, we compared the hypoperfused thickness of myocardium to transmural thickness in the respective segment. The normal perfusion was scored as 1, perfusion defect of < 25% as 2, perfusion defect of < 50% as 3, perfusion defect of > 50% as 4, perfusion defect of > 75 % as 5. The PSI was equal to sum of scores divided by sum of 16 (apex excluded). For assessment of myocardial viability, additional Gadovist was administered to reach a total dose of 0.15 mmol/kgBW. MR sequence parameters were as follows: GRE STIR (Inversion Recovery), TR 5.1 msec, TE 1.4 m sec, flip angle 300, NEX 2, bandwidth 25 kHz, TI 200 m sec. Matrix 224x160, FOV 400x400, auto-triggering end-diastolic delayed time (75% R-to-R cardiac cycles). Slice thickness 12 mm, slice spacing 6 mm. The %LGE was calculated using a special software (Report Card 3.6, Advantage Work Station AW 4.3, GE Healthcare).

Statistical Analysis

Calculation of sample size was based on the results of the largest published study²⁴, which showed an improvement of LVEF of 5.5 % after stem cell therapy. With the t-test formulation for 2 paired groups, i.e.: $n = [SDiff^2 (z\alpha + z\beta)^2]/d^2$; the required number of patients was 19 to get a power 90% (or $\beta = 0.1$, $z\beta = 1.28$); or 14 to get a power of 80% (or $\beta = 0.2$, $z\beta = 0.84$).

Continuous variables were presented as means + SD. Categorical variables were compared with use of the chi-square test or Fisher's exact test. Kolmogorov-Smirnov test was used to assess whether all variables had normal distribution. A paired t test was used to compare LVEDV, LVESV, LVEF, WMSI, PSI, and %LGE, pre and post stem cell therapy. Pearson's and Spearman-rho's correlation tests were used to assess whether there was any correlation between two numerical variables. Statistical significance was assumed for p-values of less than 0.05.

RESULTS

The characteristics of the studied population are shown in **Table 1**.

During intracoronary PBSCs injection there were no procedural complications, such as ventricular arrhythmia, visible thrombus formation, distal embolization, injury of the coronary artery associated with the cell infusion catheterization procedure or elevation of CK-MB of more than two-times. After PBSCs' injection, all patients has the myocardial blush grade III (**Table 2**). The result of culture was negative for bacterial and fungal infection in all samples. All patients had no in-stent or persistent restenosis at 6-month angiographic follow up.

Figures 1 to 3 illustrate cardiac MRI of a typical patient who already had significant LV remodeling at baseline. The large bulging in the anterior wall both during diastole and systole disappeared at 3 months. The LVEF increased from 44% at baseline to 58% at 3 months (**Figure 1**). There was also remarkable improvement in perfusion (**Figure 2**) and viability (**Figure 3**) 3 months after stem cell therapy. Figure 4 shows cardiac MRI short axis views from another patient who exhibited remarkable improvement in viability at 3 months which was even more improved at one year. %LGE reduced from 28.7% (at baseline) to 24.5% (at 3 months) and 16.9% (at one year). This was also accompanied by significant improvement of LVEF from 42% (at baseline) to 57% (at 3 months) and 57% (at one year).

Table 1. Patients' characteristics

Age (year) (mean \pm SD)(range)	55.4 \pm 6.9 (50-65)
Gender (male,%)	17 (94.4)
Diabetes [n,(%)]	3 (16.7)
Hypertension [n,(%)]	3 (16.7)
Smoker [n, (%)]	10 (55.6)
Dyslipidemia [n,(%)]	14 (77.8)
Obesity [n,(%)]	7 (38.9)
Infarct related artery: LAD (ostial or proximal)/LCX/RCA(n)	18/0/0
Single/double/triple vessel disease [n,(%)]	12(66.7)/5(27.8)/1(5.5)
Time to PCI (days) (mean + SD)	11.2 \pm 3.5
Time to stem cell injection (days) (mean \pm SD)	18.2 \pm 3.5
Number of mononuclear cells injected (mean \pm SD)	29,859,795 \pm 22,442,839
Number of CD34 \downarrow + cells injected (mean \pm SD)	1,410,002 \pm 1,205,085
Number of CD45+ cells injected (mean \pm SD)	192,023 \pm 44,026
TIMI III flow post stem cell injection [n,(%)]	
- hGr. III myocardial blushing post stem cell injection [n,(%)]	18 (100)
- CK-rise of >2 times normal [n,(%)]	18 (100)
- Other treatment [n,(%)]	0 (0)
- aspirin & clopidogrel	18 (100)
- ACE inhibitor/All receptor blocker	8 (47.0)
- β -blocker	12 (70.6)
- statin	16 (94.1)
- glycoprotein IIb/IIIa inhibitor	17 (100)
- erythropoetin	17 (100)

All patients were at NYHA Class 1 at the end of follow-up. During a follow-up procedure of 21.1 \pm 5.5 months (range 12 to 30 months) there was no death, no re-infarction, no target lesion revascularization nor re-hospitalization for heart failure (**Table 2**).

Paired cardiac MRI demonstrated no change of LVEDV at 3 months (LVEDV at baseline 110.1 \pm 31.1 ml versus 117.1 \pm 30.3 ml at 3 months, $p = 0.193$), but it increased significantly to 139.8 \pm 45.0 at one year ($p < 0.01$ versus LVEDV at baseline and at 3 months). Likewise LVESV did not change at 3 months, being 61.6 \pm 23.1 ml at baseline and 56.6 \pm 24.0 ml at 3 months ($p = 0.123$), but it increased to 69.6 \pm 35.3 at one year ($p = 0.009$ versus LVESV at baseline and $p = 0.288$ versus LVESV at 3 months). Despite this, LVEF improved significantly from 45.2 \pm 7.9 % at baseline to 52.4 \pm 8.8% at 3 months ($p = 0.001$). It remained stable

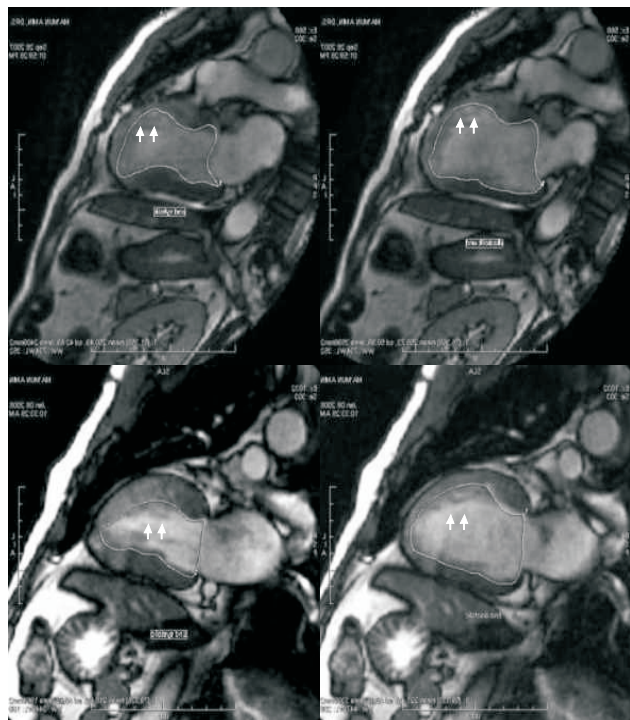


Figure 1. CMR LV functional studies at baseline (panel A: at diastole and panel C, at systole) and at 3 months (panel B, at diastole and D, at systole). Note that at baseline during diastole and systole there was bulging of the anterior wall with akinetic motion in that segment. After 3 months, bulging disappeared both during diastole and systole. At systole there was normal systolic wall thickening. Ejection fraction (LVEF) was 44% at baseline and was normalized (58%) at 3 months

at one year (LVEF $52.2 \pm 9.4\%$; $p = 0.002$ versus LVEF at baseline and $p = 0.158$ versus LVEF at 3 months). There was also improvement in WMSI which decreased significantly from 1.57 ± 0.21 at baseline to 1.37 ± 0.22 at 3 months ($p = 0.000$). It did not change at one year (WMSI 1.42 ± 0.22 ; $p = 0.048$ versus WMSI at baseline and $p = 0.426$ versus WMSI at 3 months). Significant improvement in perfusion was also noted, indicated by a decline in PSI from 1.71 ± 0.40 at baseline to 1.42 ± 0.30 at 3 months ($p = 0.003$). Although PSI further declined at one year, the difference did not reach statistical significance compared to PSI at 3 months (PSI at one year 1.24 ± 0.26 ; $p = 0.000$ versus PSI at baseline and $p = 0.179$ versus PSI at 3 months). The decline of %LGE from $25.8 \pm 7.9\%$ at baseline to $20.0 \pm 5.2\%$ at 3 months and to $16.5 \pm 4.6\%$ at one year ($p = 0.000$ for %LGE at baseline versus %LGE at 3 months; $p = 0.001$ for %LGE at baseline versus %LGE at one year; $p = 0.020$ for %LGE at 3 months versus %LGE at one year) indicated significant improvement in viability (**Table 2**).

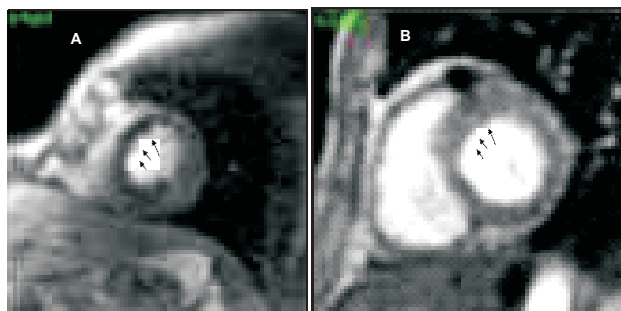


Figure 2. CMR adenosine perfusion imaging at baseline (panel A) and at 3 months (panel B). Note the underperfused area in the septal wall at baseline (black area, arrows). After 3 months, there was remarkable improvement in perfusion and wall thickness (grey area, arrows)

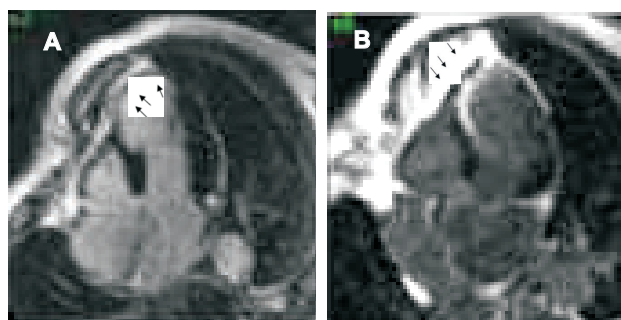


Figure 3. Myocardial delayed enhancement (MDE) in 4-chamber view at baseline (panel A) and at 3 months (panel B). Note the area of transmural infarction at the apex and septal walls at baseline (grey area, arrows). After 3 months, there was remarkable improvement in viability (black area, arrows)

There was no correlation between the level of CD34+, CD 45+, other cell subtypes (CD4+, CD8+, CD14+, CD19+, CD20+, or CD56+) as well as total number of PBSCs injected to the changes of LVEDV, LVESV, LVEF, WMSI, PSI, and %LGE ($p > 0.05$).

DISCUSSION

Experimental animal and clinical studies demonstrated that in patients with recent ST-elevation myocardial infarction, bone-marrow derived stem cells delivered by intracoronary infusion or mobilized by means of systemic administration of stimulating factors (i.e. cytokines such as G-CSF) have been shown to home to the necrotic tissue, engraft in the border zone of infarct, replicate, induce angiogenesis and myogenesis and may diminish significantly the infarct size and the extent of LV remodeling and promote a significant recovery of both global and regional cardiac function.²⁵ However, the notion that bone marrow-derived stem cells can trans-differentiate into

Table 2. Primary and secondary endpoints

Primary endpoint				
- Combined endpoints of death, myocardial infarction, stroke, arrhythmias or re-hospitalization for worsening heart failure	0	0	0	--
Secondary Endpoints (Clinical)*				
- Death	0	0	0	--
- Re-infarction	0	0	0	--
- Stroke	0	0	0	--
- Rehospitalization for heart failure	0	0	0	--
- Angiographic restenosis+	0	--	0+	--
- Target vessel revascularization	0	0	0	--
Secondary Endpoints (MRI)				
- LVEDV (ml)	110.1 ± 31.1	117.1 ± 30.3	139.8 ± 45.0	*0.193 ‡0.003 †0.002
- LVESV (ml)	61.6 ± 23.1	56.6 ± 24.0	69.6 ± 35.3	*0.123 ‡0.288 †0.009
- LVEF (%)	45.2 ± 7.9	52.4 ± 8.8	52.2 ± 9.4	*0.001 ‡0.002 †0.158
- WMSI	1.57 ± 0.21	1.37 ± 0.22	1.42 ± 0.22	*0.000 ‡0.048 †0.426
- PSI	1.71 ± 0.40	1.42 ± 0.30	1.24 ± 0.26	*0.003 ‡0.000 †0.179
- %LGE	25.8 ± 7.9	20.0 ± 5.2	16.5 ± 4.6	*0.000 ‡0.001 †0.020

LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume, WMSI, wall motion score index; PSI, Perfusion score index; %LGE, percentage late gadolinium enhancement; # Mean follow up period was 21.1 ± 5.5 months (range 12-30 months); *p value = baseline compared to 3 months; ‡p value = baseline compared to one year; †p value = 3 months compared to one year; +angiography done at 6-month

cardiomyocytes has been challenged by recent studies, which showed that only a very low level of transplanted stem cells transdifferentiated into cardiomyocytes.^{26,27} Therefore, the functional benefits of protection against LV remodeling and preservation of LV function in the absence of myocardial regeneration after stem cell implantation is likely attributed to an increased angiogenesis.²⁶⁻²⁸

During AMI, an increase of endothelial-progenitor cells (EPCs) and a selective homing of stem cells to the damaged heart have been described.^{29,30} Although the exact role of this potential self-repair mechanism is currently unknown, it has been hypothesized that manipulating its magnitude by cytokines administration soon after necrotic injury would boost LV recovery thereafter. Animal experiments showed that G-CSF

administration is an effective stimulus to mobilize bone-marrow derived stem cells into the peripheral blood and into the infarcted area, where they will be differentiated into cardiomyocytes leading to improvement in LV function.³¹⁻³⁴ Moreover, as a cytokine, G-CSF also inhibits cardiomyocyte apoptosis,^{35,36} promotes neovascularization,³⁷ and increases the production of nitric oxide.³⁸ Therefore, G-CSF not only mobilizes stem cells or progenitor cells into the injured myocardium but also has direct cardioprotective effects, at least in the setting of animal experiments. Meta-analysis in human studies, however, showed that although the use of G-CSF alone therapy in the setting of AMI after reperfusion was feasible and safe, on a cumulative basis it failed to improve LV recovery.¹⁴ In our study we used intracoronary infusion

of PBSCs mobilized with G-CSF as this strategy has been shown to improve LV systolic function significantly.^{16,17} Even though G-CSF is known to exert a variety of pro-thrombotic effects acting on both coagulation proteins and platelets,^{39,40} in our study the drug was well tolerated and did not lead to any clinical or angiographic adverse events.

Erythropoietin (EPO) traditionally is viewed as a hematopoietic hormone. However, the presence of the EPO receptor outside the hematopoietic system (i.e., endothelial cells, neurons, trophoblast cells) prompted the search for “nonhematopoietic” effects of EPO. In the heart, EPO receptor is expressed mainly on endothelial and interstitial cells and, to lesser extent, on cardiomyocytes.¹⁸ Ancillary properties of EPO include: 1). Acutely, it has direct protection against ischemic injury, probably by inhibiting programmed cell death (apoptosis).^{19,20} 2). Over a longer time frame, it may stimulate of new vessel formation (neovascularization) through angiogenesis and vasculogenesis.²³ This may further lead to improvement of cardiac function beyond an effect on infarct size.^{21,22} 3). In the setting of AMI, it also stimulates the mobilization and functional activity of EPCs.⁴¹ No adverse events were recorded during the 30-day follow up.⁴² Increased levels of circulating EPCs were associated with reduced risk of death from cardiovascular causes in patients with confirmed coronary artery disease,⁴³ suggesting a possible protective effect of EPCs in clinical setting. This was the reason why we used EPO on top of G-CSF as base of the stem cell therapy.

Infarct expansion occurs soon after onset of coronary occlusion, and it may progress in a time-dependent manner.^{44,45} Large anterior infarcts expand more frequently and severely than small ones.⁴⁵⁻⁴⁷ In our study we recruited patients who harbored a relatively large anterior wall infarction caused by proximal or ostial LAD occlusion. The time window to PCI was 11.2 ± 3.5 days and that to stem cell injection was 18.2 ± 3.5 days after onset of AMI. At this time window there are still significant amount of potent local signals for the homing of stem cells in the target area. In fact, the plasma level of the stem cell homing factor SDF-1 is up-regulated significantly from day 3 to day 28 after AMI.^{48,49} A recent meta-analysis showed that results of stem cell therapy could be better obtained in patients with lower LVEF and if the time of stem cell administration was delayed.¹²

Meta-analysis also suggested that subjects who received intracoronary cell therapy had a significant reduction in LVESV and a trend toward reduced LVEDV, as well as improvement in LVEF, perfusion

and viability.¹⁰⁻¹² However, long-term data on the safety and benefits are scarce. The REPAIR-AMI trial showed that intracoronary infusion of bone marrow-derived progenitor cells was associated with a significant reduction of the occurrence of major adverse cardiovascular event maintained for 2 years after AMI.⁵⁰ Another study reported sustained improvement in LVEF, but myocardial viability of the infarcted area did not improve at 4 years follow up.⁵¹ Contradictory findings came from ASTAMI trial, which did not demonstrate any significant benefit of such therapy,⁵² and BOOST trial which showed that the initial improvement of LVEF after bone marrow mononuclear stem cell treatment was not sustained in long-term follow-up.⁵³

Our study showed no change in LVEDV and LVESV at 3 months, but they increased significantly at one year. However, it should be acknowledged that significant LV remodeling must have occurred in our patients, given the fact that stem cell therapy took place after a time delay of more than two weeks after onset of AMI. Despite this, there were significant improvement in global contractility (measured as LVEF), improvement in regional contractility (measured as WMSI), improvement in perfusion (measured as PSI) at 3 months and these parameters remained stable at one year. We also found significant improvement in viability (measured as %LGE) at 3 months and one year. In our study 5 (29.4%) of the patients had multi-vessel disease and we always performed complete revascularization even in non-infarct related arteries. As in angiographic follow-up none of the lesions had restenosis, none of the changes could be ascribed to recruitment of collaterals. All these findings are encouraging, especially if they could be substantiated in a larger, controlled study.

One meta-analysis¹² showed absence of correlation between the results of treatment with number of cells injected, but other meta-analysis in patients with AMI reported the contrary.^{11,13} In this study we did not find any correlation between the level of CD34+, CD 45+, other cell subtypes as well as total number of PBSCs injected to the changes of LVEDV, LVESV, LVEF, WMSI, PSI, and %LGE ($p > 0.05$) However, it is perceived the ultimate effect is influenced not only by the number or type of cells, but also by other factors such as cell environment which may affect homing and engraftment, as well as timing and method of delivery and type of patient.

The absence of any acute procedural complications (including ventricular arrhythmia, thrombus formation, distal embolization, coronary artery injury, or

myocardial damage) or long-term sequels (such as re-infarction, death, rehospitalization for heart failure) was encouraging in terms of the safety and feasibility of the current stem cell transplantation strategy. An unexpectedly high rate of in-stent restenosis at culprit lesions was observed in AMI patients after bare metal stent implantation who received intracoronary injection of PBSCs mobilized with G-CSF.¹⁶ In the subsequent study of the same team they 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. They found out that G-CSF-based stem cell therapy with DES implantation was both feasible and safe, eliminating any potential for restenosis.¹⁷ In our study, we exclusively used DES and we did not encounter restenosis at 6 months angiographic follow up.

This study has several limitations. First, it was a small nonrandomized phase 1 study, therefore, we cannot completely rule out the impact of placebo effect of treatment. However, we have the impression that G-CSF and EPO-based stem cell therapy for patients with RMI is safe and feasible. Second, this study was not designed to allow the determination the optimal cell type or dose of PBSCs, the dose of G-CSF and/or EPO and to identify any potential clinical predictors for clinical improvements. No conclusion can be drawn whether the beneficial effects could be attributed to G-CSF, EPO, or both agents. Third, we did not perform functional tests for the injected PBSCs to correlate with the degree of clinical improvement after therapy. Future studies performed in double blind, randomized fashion, with larger number of patients, and longer follow up period are warranted.

CONCLUSION

In summary, this phase 1 study has shown the safety and feasibility of G-CSF and EPO-based stem cell therapy using intracoronary injection of PBSCs in patients with RMI presenting late after onset (2 weeks). We are optimistic that the ongoing phase 2, double blind, randomized study will further elucidate the remaining issues of this novel therapy.

ACKNOWLEDGEMENTS

The authors wish to thank Tommy Herryantho for his assistance in statistical analysis.

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