Intra-coronary Bone Marrow Mononuclear Cell Transplantation in Patients with ST-Elevation Myocardial Infarction: A Randomized Controlled Study

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Background: *Stem cell transplantation is a potential treatment to improve left ventricular ejection fraction (LVEF) after ST elevation myocardial infarction (STEMI). However, the outcomes still are controversial.*
Objective: *To determine the 6-month LVEF of the patients who underwent intra-coronary bone marrow mononuclear cell (BMC) transplantation in patients with STEMI compared with controlled subjects.*
Material and Method: *After successful percutaneous coronary intervention (PCI) in STEMI patients who had LVEF was less than 50% were randomized to intra-coronary BMC transplantation or control. Bone marrow aspiration of 100 cc was performed in the morning. After cell processing for three hours, the suspension of BMC about 10 cc were infused to infracted area using standard PCI technique. Balloon occlusion for three minutes was performed during cell infusion. Cardiac magnetic resonance imaging was used to determine LVEF, scar volume and LV volume before and six-month follow-up.*
Results: *Between September 2006 and July 2008, 23 patients (11 in BMC group and 12 in control group) were enrolled. Mean BMC count before transplant was 420 x 10^6 cell with 96% viability. At six-month follow-up, New York Heart Association function class significantly improved in both groups (2.3 $\pm$ 0.6 to 1.2 $\pm$ 0.4 for BMC and 2.3 $\pm$ 0.7 to 1.3 $\pm$ 0.5 for control group) but no difference was seen between groups. However, scar volume, wall motion score index, and LVEF did not show improvement after six months in both groups (33.7 $\pm$ 7.7 to 33.5 $\pm$ 7.6 for BMC and 31.1 $\pm$ 7.1 to 32.6 $\pm$ 8.3 for control group). No complication was observed during the procedure.*
Conclusion: *BMC transplantation intra-coronary in patients with STEMI in KCMH was feasible and safe but LVEF improvement could not be demonstrated.*

Keywords: *Intracoronary, Bone marrow mononuclear cell transplantation, ST elevation myocardial infarction*

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Stem cell therapy is an emerging and potential entity for treating cardiovascular disease particularly myocardial infarction. This proposes would like to repair or develop angiogenesis into the infracted or ischemic area. Because of the advance of stem cell knowledge, many reports tried to implant stem cells into the heart muscle either via intra-coronary or direct injection for myocardial regeneration[1-15]. Some of these trials demonstrated improvement of left ventricular (LV) ejection fraction (EF) whereas many

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did not. Because of different technique, different cell type, different inclusion criteria, or different method of evaluation, the outcomes may be different.

At King Chulalongkorn Memorial Hospital, the authors reported earlier the safety and some improvement of LV using bone marrow mononuclear (BMC) intra-coronary transplantation[16]. However, the study did not have control subjects. The improvement may be caused by revascularization and some medical treatment for myocardial infarction. The present study was conducted to determine the outcomes of intra-coronary bone marrow mononuclear cell (BMC) transplantation in STEMI patients compared with the control group who received standard therapy for myocardial infarction.

Material and Method

This was the single centre, randomized control study by simple random technique to determine the improvement of LV EF using cardiac magnetic resonance (CMR) imaging. The secondary objects were to determine the improvement of LV EF by echocardiography, the regional wall motion score index and safety of BMC intra-coronary transplantation. The authors enrolled the patients who had a history of STEMI with LVEF less than 50% and demonstrated regional wall motion abnormality by echocardiography. The patient had to have successful angioplasty with or without stent implantation for infarct related artery. Echocardiogrpahy and CMR were performed under blind information who received stem cell or not to evaluate LVEF and regional wall motion abnormality prior stem cell infusion, three, and six months after BMC transplantation. Coronary angiography (CAG) and LV angiography were performed before and six months after transplantation. The authors excluded the patients who had cardiogenic shock, severe congestive heart failure (function class 4), impaired renal function (creatinine > 1.8 mg/dl), pregnancy, and other severe co-morbid disease with life expectancy less than one year. All patients had to sign informed consent prior enrollment. The present study was approved by the ethic committee of the Faculty of Medicine, Chulalongkorn University.

BMC preparation

One hundred ml of bone marrow from the iliac crest was harvested in the morning under local anesthesia with conscious sedation and sent immediately to the cell-processing laboratory at the National Blood Centre, the Thai Red Cross Society. Mononuclear cells were isolated by density gradient centrifugation (Isoprep®). After two washing steps with saline+2% autologous serum, cells were suspended in 10 ml of saline+2% autologous serum. Cells population including total mononuclear cells, CD34+ cells, and CD133+ cells were analyzed using flow cytometer as well as the cell viability study before transplantation.

Cell transplantation technique

Standard percutaneous coronary intervention (PCI) procedure was performed to transplant BMC immediately after cell processing. Over-the-wire balloon was inflated in the infarct related artery to stop-flow and then slowly infused 3.3 ml of cell suspension through the wire lumen into the infarction area. Balloon was occluded for three minutes and three times for cells infusion. Coronary angiography was done before finishing the procedure.

Statistical analysis

The Intercooled STATA version 10.0 is used for data analysis. The baseline characteristics for nominal variables were expressed in number and percent and continuous variables are expressed as mean $\pm$ SD. Fisher's exact Chi-square test and student unpaired t-test are used to compare the data between the two groups. Paired t-test is used to compare pre and post at six-month. P-value less than 0.05 is considered statistically significant.

Results

Between October 2005 and July 2008, 23 patients (11 in the BMC group and 12 in the control group) were enrolled. The baseline characteristics are shown in Table 1. Mean age, sex, atherosclerosis risk factors and area of myocardial infarction were not different in both groups. Duration of myocardial infarction to randomize was 57.2 $\pm$ 122.8 days in the BMC group and 45.3 $\pm$ 37.2 days in the control group ($p = 0.763$). Functional class and biomarker NT pro-BNP as well as LV EF were also the same in both groups. In the BMC group, average total volume of bone marrow harvest was 123 $\pm$ 15.7 ml. After cell processing, average total mononuclear cell was 420 $\pm$ 221 x 10^6 cells with cell viability about 95.9%. These cells contained of 2.14% of CD34+ and 1.2% of CD133+. After 6-month follow-up, the restenosis rate was 60% and 50% in the BMC and control group respectively. Symptom and biomarker NT pro-BNP of congestive heart failure were improved when compared with the baseline (Table 2).

Table 1. Baseline characteristics of the patients

Baseline characteristics	Stem cell	Control	p-value
Number of patients (n)	11	12	
Age (years)	52.0 ± 12.9	54.1 ± 14.7	0.711
Sex: male (%)	81.8	83.3	1.000
Diabetes (%)	18.2	33.3	0.640
Hypertension (%)	36.4	33.3	1.000
Dyslipidemia (%)	81.8	50.0	0.193
Smoking (%)	54.6	66.7	0.680
Duration of MI prior to randomize (d)	57.2 ± 122.8	45.3 ± 37.2	0.763
NYHA FC	2.3 ± 0.6	2.3 ± 0.7	0.825
1 (%)	9.1	8.3	1.000
2 (%)	54.6	50.0	
3 (%)	36.4	41.7	
Infarct related artery: LAD (%)	81.8	100	0.217
Diseased vessel			0.187
1 VD (%)	63.6	91.7	
2 VD (%)	27.3	8.3	
3 VD (%)	9.1	0	
LVEF by CMR (%)	33.7 ± 7.6	31.1 ± 7.1	0.397
Infarct volume (ml)	43.5 ± 17.6	45.0 ± 15.9	0.837
HsCRP	3.48	2.97	0.084
NT pro BNP (ng/ml)	2,293.0 ± 1,560.0	1,871.0 ± 1,541.0	0.530
Medication			
ASA (%)	100	100	1.000
Clopidogrel (%)	100	91.7	1.000
BEtablocker (%)	54.6	75.0	0.400
ACEI (%)	81.8	75.0	1.000
Statin (%)	90.9	100	0.478
Stent: DES (%)	18.2	16.7	1.000
Restenosis after 6 month (%)	60.0 (6/10)	50.0 (6/12)	0.691
BM aspiration volume (ml)	123.0 ± 15.7		
Total mononuclear cell (x10^6)	420.0 ± 221.0		
CD 34+ (%)	2.14 ± 1.1		
Total amount of CD 34+ (x10^6)	8.0 ± 4.5		
CD 133+ (%)	1.2 ± 0.6		
Total amount of CD 133+ (x10^6)	4.2 ± 1.9		
Viability (%)	95.9 ± 2.6		

MI = myocardial infarction; NYHA-FC = New York Heart Association functional class; LAD = left anterior descending artery; LVEF = left ventricular ejection fraction; CMR = cardiac magnetic resonance imaging; HsCRP = high sensitive C reactive protein; NT pro-BNP = N terminal pro-brain natriuretic peptide; ACEI = angiotensin converting enzyme inhibitor; DES = drug eruting stent; BM = bone marrow

Table 2. NYHA FC and biomarker pre and 6 month post

	Stem cell	Control	p-value
Number of patients (n)	11	12	
NYHA FC			
Pre	2.3 ± 0.6	2.3 ± 0.7	0.825
6-months	1.2 ± 0.4	1.3 ± 0.5	0.708
Change from baseline	1.1 ± 0.5	1.1 ± 0.8	0.979
p-value	<0.001	<0.001	
NT pro BNP			
Pre	2,293.0 ± 1,560.0	1,871.0 ± 1,541.0	0.530
6-months	1,180.0 ± 1,571.0	902.0 ± 822.0	0.515
Change from baseline	1,114.0 ± 1,579.0	969.0 ± 1,598.0	0.833
p-value	0.041	0.072	

NYHA-FC = New York Heart Association functional class; NT pro-BNP = N terminal pro-brain natriuretic peptide

However, if comparing the BMC group with the control group, there were no statistical significant differences. The LV EF, scar volume, LV end diastolic volume, LV end systolic volume and wall motion score index were not different at 6-months in both groups (Table 3). During the present study period, no adverse cardiac events were observed.

Discussion

The present study showed the improvement of symptom and biomarker for congestive heart failure in both BMC and the control group. This may due to the standard treatment of myocardial infarction without any change or related to LV function. When looking into the LV function, the authors did not find any improvement of LV systolic function measure by CMR or echocardiography. This finding is consistent with some previous reports[4,13] but different from the REPAIR-MI[14]. However, the increase of LV EF in REPAIR-MI was very little and the authors do not know if this slight improvement can be translated into clinical outcomes. LV scar volume in the present study

Table 3. LV function pre and at 6 month after randomized

	Stem cell	Control	p-value
Number of patients (n)	11	12	
LVEF by CMR (%)			
Pre	33.7 ± 7.7	31.1 ± 7.1	0.397
6-months	33.5 ± 7.6	32.6 ± 8.3	0.776
Change from baseline	-0.2 ± 7.7	1.5 ± 6.1	0.566
p-value	0.939	0.415	
LVEF by Echo (%)			
Pre	36.3 ± 5.7	42.2 ± 6.1	0.114
6-months	38.0 ± 9.4	42.0 ± 8.7	0.252
Change from baseline	1.7 ± 11.9	-0.1 ± 9.9	0.709
p-value	0.686	0.953	
LVEF by angiography (%)			
Pre	41.5 ± 5.6	38.6 ± 7.5	0.315
6-months	36.6 ± 13.8	38.1 ± 13.1	0.709
Change from baseline	-4.1 ± 12.1	-0.5 ± 10.9	0.471
p-value	0.312	0.876	
LVEDV (ml)			
Pre	154.0 ± 26.0	189.0 ± 70.0	0.127
6-months	162.0 ± 31.0	177.0 ± 45.0	0.366
Change from baseline	8.3 ± 24.3	-12.2 ± 58	0.298
p-value	0.285	0.049	
LVESV (ml)			
Pre	103.0 ± 25.0	138.0 ± 68.0	0.120
6-months	108.0 ± 29.0	113.0 ± 44.0	0.540
Change from baseline	5.9 ± 22.2	-19.8 ± 65.8	0.233
p-value	0.399	0.321	
Scar volume (ml)			
Pre	43.5 ± 17.6	43.1 ± 15.2	0.837
6-months	34.7 ± 17.2	38.4 ± 12.2	0.574
Change from baseline	-8.8 ± 14.1	-4.7 ± 10.5	0.513
p-value	0.065	0.168	
Scar to LV volume (%)			
Pre	44.3 ± 17.6	44.6 ± 18.7	0.950
6-months	34.7 ± 17.2	44.1 ± 15.7	0.651
Change from baseline	-4.0 ± 13.6	-0.5 ± 9.6	0.513
p-value	0.374	0.384	
RWMA score index			
Pre	1.8 ± 0.3	2.0 ± 0.3	0.455
6-months	1.7 ± 0.2	2.0 ± 0.3	0.098
Change from baseline	-0.07 ± 0.3	-0.02 ± 0.4	0.751
p-value	0.526	0.909	

LVEF = left ventricular ejection fraction; CMR = cardiac magnetic resonance imaging; LVEDV = left ventricular end diastolic volume; LVESV = left ventricular end systolic volume; RWMA = regional wall motion abnormality

had a trend to reduce but did not reach statistical significance. This reduction may be due to the shrinkage of myocardial edema that occurred during myocardial infarction. It can be detected by CMR using delay enhancement protocol. The present study cannot determine the improvement of LV systolic function. Some reasons can be explained; first, the BMC cannot maintain at the infracted area. Penicka M et al demonstrated that only 1% of BMC remained in the heart when imaged at 18-hours after transplantation[5]. Second, which cells populations are needed to transplant? Only one cell population using expansion in vitro or whole cells from bone marrow preparation are used for transplantation. Most of the studies would like to use bone marrow mononuclear cell because it is quite easy to proceed. In the present study, the authors used BMC and after cell processing, more than 90% of cells were viable. Up to now, the authors do not know which cells are suitable for transplantation. May be cytokine is needed to integrate the cells in the infarcted area and initiate the cells to trans-differentiate to cardiac myocyte. Third, when do the authors need to transplant cells; immediately, early, or late myocardial infarction. Janssen S et al reported failure to augment the recovery of global LV function using bone marrow-derived stem cell transplant one day after myocardial infarction[13]. If the authors transplant cells too late, some cytokines will not persist for cells homing. Intharaphet P et al demonstrated the higher number of endothelial progenitor cells in intracoronary in acute coronary syndrome when compared with chronic stable angina and control subjects[17]. This finding supports the homing theory after cell injury. Finally, which is the best route to transplant these cells? Intracoronary seems to be the simplest technique as routinely performed in daily practice. Even the present study could not determine the improvement of LV systolic function, but it can be performed safely.

Conclusion

BMC transplantation intra-coronary in patients with STEMI in KCMH is feasible and safe but LVEF improvement could not be demonstrated. Many questions about stem cell therapy for myocardial infarction require answering.

Potential conflicts of interest

None.

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การรักษาผู้ป่วยกล้ามเนื้อหัวใจตายเฉียบพลันชนิด ST-elevation โดยการใช้ bone marrow mononuclear cell ฉีดเข้าในหลอดเลือดหัวใจ: การศึกษาแบบสุ่มที่มีกลุ่มควบคุม

สุพจน์ ศรีมหาโชตะ, สมนพร บุณยะรัตเวช, ไพโรจน์ พฤกษ์พัฒนาพิพัฒน์, สมใจ วังศุภชาติ, มนรวีร์ ทุมโฆษิต, อุดมศักดิ์ บุญวรเศรษฐ์, ธัญญพงษ์ ณ นคร, ธานินทร์ อินทรกำธรชัย, ภาวิณี คุปตวินธุ, สุนิสา โพธิ์งาม, เอมอร แสงศิริ, มัณนา โพธิ์ศรี, ยี่สุ่น สุขเสรี, ถนอม บรรณประเสริฐ, ถาวร สุทธิไชยากุล

ภูมิหลัง: *การใช้เซลล์ต้นกำเนิดเป็นความหวังในการรักษาผู้ป่วยกล้ามเนื้อหัวใจตายเฉียบพลันชนิด ST elevation ที่มีการทำงานของหัวใจลดลง อย่างไรก็ตามผลการรักษายังไม่มีข้อสรุปแน่ชัด*

วัตถุประสงค์: *เพื่อศึกษาการเปลี่ยนแปลงของการทำงานของหัวใจที่ 6 เดือนภายหลังการให้การรักษาด้วย bone marrow mononuclear cell (BMC) ฉีดเข้าในหลอดเลือดหัวใจในผู้ป่วยกล้ามเนื้อหัวใจตายเฉียบพลันชนิด ST-elevation เปรียบทียบกับกลุ่มควบคุม*

วัสดุและวิธีการ: *หลังจากที่ทำบอลลูนขยายหลอดเลือดในผู้ป่วยกล้ามเนื้อหัวใจตายเฉียบพลันชนิด ST elevation และมีการทำงานของหัวใจน้อยกว่า 50% โดยสุ่มผู้ป่วยเข้าในกลุ่มที่ได้รับการรักษาด้วย bone marrow mononuclear cell (BMC) ฉีดเข้าในหลอดเลือดหัวใจหรือกลุ่มควบคุม ผู้ป่วยจะได้รับการเจาะไขกระดูกจำนวน 100 มล. ในตอนเช้า และส่งไปทำการแยก mononuclear cell โดยใช้เวลา 3 ชั่วโมง ซึ่งจะได้ของเหลวที่มีเซลล์ปริมาตร 10 มล. เพื่อที่จะฉีดเข้าหลอดเลือดหัวใจโดยใช้วิธีการปกติของการทำบอลลูนขยายหลอดเลือด ขณะที่ฉีดเซลล์เข้าสู่กล้ามเนื้อหัวใจบอลลูนจะถูกขยายออกเป็นเวลา 3 นาที การทำงานของหัวใจ ปริมาณของกล้ามเนื้อที่ตายจะตรวจโดยการใช้คลื่นแม่เหล็กโดยเปรียบเทียบก่อนทำและที่ 6 เดือนหลังจากทำการฉีดเซลล์ต้นกำเนิดและเปรียบเทียบกับกลุ่มควบคุม*

ผลการศึกษา: *จากเดือนกันยายน พ.ศ. 2550 ถึง กรกฎาคม พ.ศ. 2551 ผู้ป่วย 23 ราย (11 รายในกลุ่ม BMC และ 12 รายในกลุ่มควบคุม) ได้เข้าสู่การศึกษา ค่าเฉลี่ยจำนวน BMC เท่ากับ 420 x 10^6 เซลล์ โดยที่ 96% เป็นเซลล์ที่ยังมีชีวิต ภายหลังติดตามการรักษาที่ 6 เดือน พบว่า function class ดีขึ้นอย่างมีนัยสำคัญทั้ง 2 กลุ่ม (2.3 $\pm$ 0.6 to 1.2 $\pm$ 0.4 สำหรับกลุ่ม BMC and 2.3 $\pm$ 0.7 to 1.3 $\pm$ 0.5 สำหรับกลุ่มควบคุม) แต่ไม่พบความแตกต่างกันระหว่างกลุ่ม อย่างไรก็ตาม ไม่พบความแตกต่างของ LVEF เมื่อติดตามไป 6 เดือนของทั้งสองกลุ่ม (33.7 $\pm$ 7.7 to 33.5 $\pm$ 7.6 สำหรับกลุ่ม BMC and 31.1 $\pm$ 7.1 to 32.6 $\pm$ 8.3 สำหรับกลุ่มควบคุม) เช่นเดียวกับ scar volume และ wall motion score index ไม่พบภาวะแทรกซ้อนใด ๆ เกิดขึ้นระหว่างที่ทำหัตถการ*

สรุป: *การใช้ BMC ในการฉีดเข้าหลอดเลือดหัวใจในผู้ป่วยกล้ามเนื้อหัวใจตายเฉียบพลันชนิด ST-elevation สามารถทำได้และมีความปลอดภัย แต่ไม่สามารถแสดงให้เห็นถึงการเพิ่มขึ้นของ LVEF*