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Safety and efficacy of intracoronary *h*ypoxia-precond*i*tioned bone marrow mono*n*uclear cell *a*dministration for *a*cute *m*yocardial *i*nfarction patients: The CHINA-AMI randomized controlled trial

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ABSTRACT

Background: Pre-clinical studies have shown that hypoxia preconditioning can enhance stem cell therapeutic potential for myocardial repair. We sought to investigate the safety and feasibility of intracoronary administration of hypoxia-preconditioned bone marrow mononuclear cells (HP-BMCs) for acute ST segment elevation myocardial infarction (STEMI).

Methods: We randomized 22 patients with acute STEMI to receive intracoronary administration of normoxia bone marrow mononuclear cells (N-BMCs) (n = 11) or HP-BMCs (n = 11) following successful reperfusion. Another 14 patients receiving standard therapy were recruited as control (n = 14).

Results: There were no differences in the occurrence of major adverse cardiovascular events at 30 days and 1 year among three groups. There were significant improvement in the change of left ventricular end-diastolic volume (LVEDV) and end-systolic volume (LVESV) in HP-BMC group both at 6 and 12 months compared with N-BMCs or control group ($P < 0.05$). No differences were observed in the change of left ventricular ejection fraction (LVEF), or wall motion score index (WMSI) among three groups. Nevertheless, WMSI was improved in HP-BMCs and N-BMC group ($P < 0.05$, within group), but not in control. The ratio of myocardial perfusion defect determined by SPECT was significantly decreased in HP-BMCs and N-BMC groups at 6 months compared with baseline ($P < 0.05$, within group), but no significant differences were observed among three groups.

Conclusions: Our results provide the first-in-man evidence that intracoronary administration of HP-BMCs following acute MI appears to be safe and feasible. These results provide the basis for future prospective randomized clinical trials in a larger patient cohort.

Clinical trial registration information: NCT01234181 (http://clinicaltrials.gov/ct2/show/NCT01234181?term=NCT01234181&rank=1).

1. Introduction

Autologous bone marrow mononuclear cell (BMC) administration has been investigated as a potential novel therapy to improve left ventricular (LV) function, reverse remodeling and reduce myocardial scarring in patients with acute myocardial infarction (MI) [1]. Nevertheless some recent randomized controlled trials [2,3] and meta-analysis [4,5] have failed to demonstrate any consistent improvement in LV function or infarct size. This highlights the need for adjunctive strategies to further enhance the therapeutic efficacy of current stem cell therapy for acute MI. Prior experimental studies suggest that poor cell engraftment and survival of the transplanted BMCs are major hurdles to the development of effective cell-based therapy [6–8]. Exposure of the stem cells to low oxygen tension, i.e., hypoxia preconditioning, has been shown to improve survival of stem

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cells after transplantation [9] and enhance their therapeutic potential for myocardial repair [10]. Indeed our previous experimental studies [11,12] demonstrated that hypoxia preconditioning enhances BMC survival, migration, and angiogenesis, and thus improves their therapeutic efficacy. Nevertheless, there are no human data on the safety and therapeutic efficacy of BMCs following hypoxia preconditioning in the treatment of acute MI.

In this phase 1, prospective, randomized and double-blind controlled trial, we sought to determine the safety and feasibility of intracoronary administration of autologous hypoxia-preconditioned BMCs (HP-BMCs) in patients with acute ST segment elevation MI following successful reperfusion.

2. Methods

2.1. Study design and patient enrollment

From February 2011 to March 2012, patients aged between 18 and 75 years old were eligible for the study if they presented with an acute ST segment elevation MI that had been successfully reperfused by means of primary percutaneous coronary intervention (PCI) with stent implantation or thrombolysis; and had a substantial residual LV regional wall motion abnormality (defined as wall motion score index (WMSI) > 1) evident on transthoracic echocardiogram. Patients were excluded from study if they had any of the following: life expectancy < 1 year, sepsis, hemodynamic instability, moderate–severe renal impairment (glomerular filtration rate < 50 mL/min/1.73 m^2), New York Heart Association Class IV heart failure on intravenous inotropic therapy, need for further coronary revascularization or significant valvular heart disease. The research protocol was approved by the Ethics Committee of Human Research of Second Affiliated Hospital of Zhejiang University, and all participants provided written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. The protocol has been registered at ClinicalTrials.gov (Trial identifier: NCT01234181).

2.2. Study protocol

Twenty two patients were randomly assigned to the normoxia BMC group or hypoxia-preconditioned BMC group in a 1:1 ratio using a randomization number table. Patients randomized to each group received 10×10^7 BMCs that had been cultured for 24 h in a normoxic or hypoxic environment. Another 14 patients received standard treatment as control. Patients, study coordinators, and the investigators who were responsible for patient assessment were blinded to the randomization process. Baseline evaluation in three groups including echocardiography and single-photon emission computed tomographic (SPECT) perfusion study, performed at 3–5 days following PCI, and repeated at 6 and 12-month follow-ups. Arrhythmia was assessed after infusion, at 1 month, 6 months and 12 months using a Holter monitor.

2.3. Preparation and administration of BMCs

At day 5 after PCI, BMCs were harvested from patients by an experienced doctor via posterior iliac crest puncture under local anesthetic. A total of 80–100 mL of bone marrow blood was aspirated. BMCs were isolated by Ficoll density gradient centrifugation as previously described [13,14]. BMCs were washed twice in phosphate-buffered saline with 0.5% human serum albumin. The final cell suspension contained 10×10^7 BMCs in 10 ml of saline. The BMCs were analyzed by fluorescence-activated cell sorting using anti-CD34, anti-CD133, anti-CD309, anti-CD117, anti-CD29, anti-CD166 and anti-mSca-1 (BD Pharmagin, San Jose, CA). Cell viability was assessed by Trypan blue staining.

BMCs were plated on serum free culture medium (StemPro® SFM XenoFree complete medium, GIBCO Invitrogen, Carlsbad, CA, USA) and cultured for 24 h. Normoxia BMCs were incubated under normoxic conditions (37 °C, 21% O_2, 5% CO_2). Hypoxia preconditioning treatment was achieved by placing cells in a well-characterized, finely controlled ProOx-C-chamber system (BioSpherix, Redfield, NY, USA) for 24 h. The O_2 concentration in the chamber was maintained at 0.5%, with a residual gas mixture composed of 5% CO_2 and balanced N_2.

BMCs were administrated using a stop-flow [15] technique through an over-the-wire balloon catheter (Open-sail, Guidant, USA) positioned within the stented segment of the infarct-related artery. A total of 10×10^7 normoxia or hypoxia-preconditioned BMCs were infused into the infarct related myocardium. All patients received unfractionated heparin to achieve an activated clotting time >300 s during the procedure.

2.4. Study endpoint

Primary endpoint was the safety of the therapy defined by the incidence of any treatment associated major adverse cardiovascular events (MACEs) including death, nonfatal MI, stroke, heart failure hospitalization or hemodynamic significant or sustained ventricular arrhythmias (>15 s as detected by 24 h Holter monitoring) within 30 days of BMC injection. The secondary safety endpoint included the occurrence of MACEs within 12 months.

Pre-specified secondary efficacy endpoints were change (6-month follow-up minus baseline) in myocardium perfusion defect ratio as determined by SPECT, and global left ventricular ejection fraction (LVEF), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV) and WMSI as measured by echocardiography.

2.5. Echocardiographic analysis

Standard M-mode, two dimensional and color Doppler echocardiography were performed [16] by a single experienced operator who was blinded to patients' treatment. The measurements included LVESV, LVEDV, WMSI and LVEF. These measurements were computed using the biplane rule methods of Simpson [17].

2.6. Cardiac SPECT scan

SPECT perfusion test was performed to identify changes in ischemic defects at rest using a standardized protocol. In brief, 740–925 MBq (20–25 mCi) 99mTc-MIBI was injected and gated myocardial SPECT performed 60 min later using a dual-detector SPECT system (Siemens E.CAM duet) equipped with low-energy high-resolution collimators. The SPECT images were gated with eight frames per cardiac cycle. Acquisition parameters were as follows: energy window 140 keV ± 10%, 180° noncircular orbit, 5.6° per step, 64 × 64 matrix, zoom ×1.45, 40 s acquisition per step.

Transaxial slices were reconstructed via back projection with a ramp filter, followed by a Butterworth filter with an order of 10 and cut-off of 0.55. Software of the Emory Cardiac Toolbox (ECTb, Emory University, Atlanta, GA, USA) was used to create polar maps and obtain other parameters. The infarct size was expressed as a percentage of the LV. The LV myocardium was divided into 20 segments, and the ratio of myocardium perfusion defect was calculated.

2.7. Statistical analysis

All statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc.). Descriptive statistics for baseline characteristics were generated for demographic variables, medical history, and laboratory data. Categorical variables were presented as frequency and percent. Continuous variables (normal distribution) were presented as mean and standard derivation or median and range (non-normal distribution). Chi-squared and ANOVA tests were used to evaluate the differences among 3 groups at baseline. Wilcoxon signed-rank test was used to evaluate the difference of non-normal distribution data among 3 groups. Within group comparisons were performed using Student's *t*-test. Fisher

exact test was used for the comparison of AEs and SAEs among three groups. For the intention-to-treat analysis of echo and SPECT variables, an analysis of covariance (ANCOVA) was performed to assess the differences between treatment groups at 6 and 12 months, after adjusting for baseline values. Specifically, values at 6 and 12 months were taken as the dependent variable and the associated baseline values and a factor for treatment were taken as independent variables. Model adequacy was assessed by examining the standardized residuals. The absolute change of each value between 6 month (12 month) and baseline were tested using ANOVA. Two-sided significance testing was used; P value < 0.05 was deemed statistically significant.

3. Results

3.1. Enrollment and baseline characteristics

From 2011 February to 2012 March, 101 patients with an acute ST segment elevation MI were screened, and 22 patients who fulfilled the inclusion criteria were recruited. They were randomly assigned to receive normoxia BMCs or hypoxia-preconditioned BMCs. Other 14 patients receiving standard treatment served as the control group (Fig. 1). In the normoxia BMC group, one patient was excluded due to the presence of thrombus in the coronary artery prior to intracoronary infusion. Successful intracoronary infusion of BMCs was performed in the remaining 21 patients. No patient was lost to follow-up during the study period and the three groups were matched for baseline clinical characteristics and concomitant pharmacologic therapy during the study, except that more patients in the hypoxia-preconditioned group were diabetic (Table 1).

3.2. Effect of hypoxia preconditioning on cell characteristics

Trypan blue staining revealed no significant difference in cell viability between the normoxia and the hypoxia-preconditioned group ($96.4 \pm 2.9\%$ vs. $98.2 \pm 1.7\%$, $P = 0.18$). The percentage of CD34+, CD133+, CD309+, CD117+, CD29+, CD166+, mSca-1+ cells was also similar in normoxia BMCs and hypoxia-preconditioned BMC groups (Table 2).

3.3. Safety endpoints

There were no differences among three groups in the primary and secondary safety endpoints with respect to the occurrence of MACEs at 30 days and 1 year. Within the first 30 days, no patient in the normoxia group, one patient in the hypoxia-preconditioned group and two patients in control group died (0/11, 0%, 1/11, 9.1%, 2/14, 14.3%, $P = 0.76$) (Table 3). No other adverse event was noted. During one-year follow-up, MACEs occurred in 3/10 (30%) patients in the normoxia group (hospitalization for heart failure, n = 3),1/11 (9.1%) patients in the hypoxia-preconditioned group (hospitalization for heart failure, n = 1; Target-vessel revascularization, n = 1) and no patient in control group (Table 3). Specifically, no patient in three groups experienced sustained ventricular tachycardia following 30 day and 1 year follow-up.

3.4. Cardiac function and remodeling

Baseline LVEF, LVEDV and LVESV were $56.9 \pm 12.4\%$, 115.2 ± 42.5 ml and 55.1 ± 32.2 ml respectively in the normoxia group, $50.9 \pm 12.9\%$, 136.9 ± 34.7 ml and 72.8 ± 32.8 ml respectively in the hypoxia-preconditioned group and $57.1 \pm 11.6\%$, 109.2 ± 19.7 ml and 48.8 ± 21.8 ml respectively in control group (Table 4). The WMSI was 1.3 ± 0.5, 1.5 ± 0.4 and 1.4 ± 0.3 in the normoxia group, hypoxia-preconditioned group and control group, respectively. There was significant improvement in change of LVEDV and LVESV in hypoxia-preconditioned group both at 6 months and 12 months when compared with normoxia group and control group, suggesting hypoxia-preconditioned BMC therapy may be more effective to postpone LV

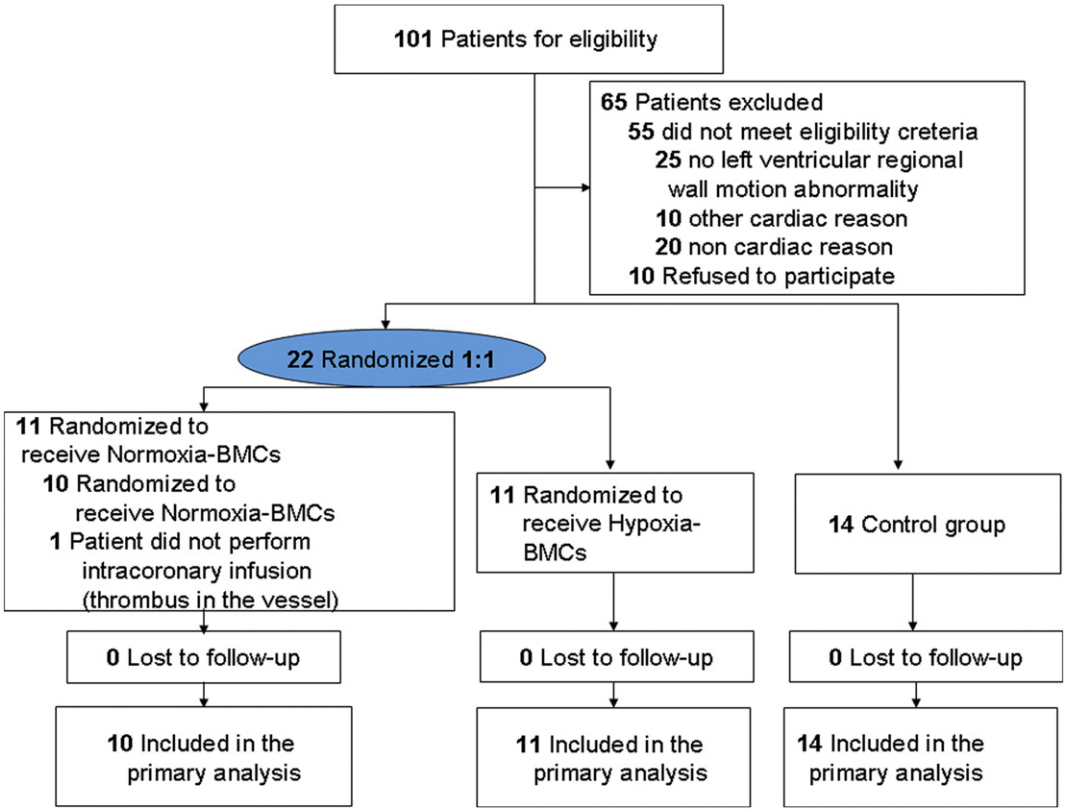

Fig. 1. Study flow chart.

Table 1
Characteristics of patients.

	Normoxia (n = 11)	Hypoxia-preconditioned (n = 11)	Control (n = 14)	*P* value
Age, years	61.2 ± 12.8	59.7 ± 11.7	60.62 ± 10.85	0.96
Female	2 (18.2)	1 (9.1)	5 (35.7)	0.26
Killip-class				
I	9 (81.8)	9 (81.8)	9 (64.29)	
II	1 (9.1)	1 (9.1)	2 (14.29)	
III	1 (9.1)	0 (0)	2 (14.29)	
IV	0 (0)	1 (9.1)	1 (7.14)	0.81
Coronary artery disease				
1-vessel	3 (27.3)	5 (45.5)	4 (28.57)	0.59
2-vessel	4 (36.4)	0 (0)	5 (35.71)	0.07
3-vessel	4 (36.4)	6 (54.6)	5 (35.71)	0.58
Infarct related artery				
Left anterior descending	8 (73.7)	5 (45.5)	7 (50.00)	0.38
Right coronary artery	2 (18.2)	5 (45.5)	5 (35.71)	0.39
Left circumflex	1 (9.1)	1 (9.1)	2 (14.29)	0.89
Average time from symptom onset to PCI	6.11 ± 2.2	6.0 ± 2.4	5.77 ± 2.63	0.95
Stent number				
0	4 (36.4)	3 (27.3)	3 (21.43)	
1	5 (45.5)	7 (63.6)	11 (78.57)	
2	2 (18.2)	1 (9.1)	0 (0)	0.41
Length of stent, mm	41.3 ± 26.3	36.4 ± 27.5	25.40 ± 5.87	0.28
Size of stent, mm	3.0 (3.0–54.0)	3.5 (3.0–4.0)	3.25 (3.00–3.50)	0.42
Blood biochemistry				
Creatine Kinase	4101.9 ± 2015.0	4395.8 ± 2876.0	3542.9 ± 2424.2	0.70
Troponin-I,	103.7	188.00	101.0	0.40
Median (range)	(84.80–309.00)	(58.92–317.00)	(33.2–159.0)	
Medical history				
Diabetes	0 (0)	5 (45.5)	2 (14.29)	0.03
Hypertension	5 (45.5)	5 (45.5)	6 (42.86)	0.99
Hyperlipidemia	5 (45.5)	3 (27.3)	2 (14.29)	0.22
Former or current smoking	7 (63.6)	10 (90.9)	7 (50.0)	0.10
Medications				
Baseline				
Aspirin	11 (100)	11 (100)	14 (100)	/
Clopidogrel	11 (100)	11 (100)	14 (100)	/
Statin	11 (100)	11 (100)	14 (100)	/
ACEI	9 (81.8)	10 (90.9)	11 (78.57)	0.70
Beta-blocker	10 (90.9)	8 (72.7)	11 (78.57)	0.54
12-month	N = 10	N = 10	N = 12	
Aspirin	10 (100)	10 (100)	12 (100)	/
Clopidogrel	10 (100)	10 (100)	12 (100)	/
Statin	10 (100)	10 (100)	12 (100)	/
ACEI	8 (80)	8 (80)	8 (66.7)	0.70
Beta-blocker	8 (80)	9 (90)	11 (91.7)	0.68

ACEI, Angiotensin-converting enzyme inhibitor; PCI, percutaneous coronary intervention
Data are means (SD), medium (range), or *n* (%) unless otherwise stated.

remodeling. However, there were no significant differences among the three groups in the change of LVEF and WMSI. Nonetheless LVEF were significantly decreased at 6 months compared with baseline in the control group (Table 4, $P < 0.05$), hypoxia-preconditioned BMC therapy showed a trend of increased LVEF. WMSI were decreased at 6 months compared with baseline in the hypoxia-preconditioned group, but not in the normoxia group ($P < 0.05$). At 12 months, WMSI were decreased both in the hypoxia-preconditioned group and the normoxia group.

Table 2
The effect of hypoxia-preconditioning on BMC product characteristics.

Characteristics	Normoxia BMCs	Hypoxia-preconditioned BMCs	*P* value
% Viability by Trypan blue exclusion, mean ± SD	96.4 ± 2.9	98.2 ± 1.7	0.18
% of CD34 cells, mean ± SD	2.0 ± 1.9	2.5 ± 2.4	0.62
% of CD133 cells, mean ± SD	0.9 ± 1.0	1.0 ± 0.9	0.17
% of CD309 cells, mean ± SD	0.2 ± 0.2	0.2 ± 0.2	0.78
% of CD117 cells, mean ± SD	1.8 ± 1.7	5.1 ± 3.6	0.10
% of CD29 cells, mean ± SD	45.2 ± 30.6	53.0 ± 12.4	0.37
% of CD166 cells, mean ± SD	3.8 ± 5.0	1.8 ± 2.5	0.42
% of mSca-1 cells, mean ± SD, (n)	0.1 ± 0.2	0.3 ± 0.3	0.20

BMCs, bone marrow mononuclear cells.

3.5. Quantitative variables of cardiac perfusion

SPECT imaging was performed to evaluate myocardial perfusion following BMC administration (Table 5). At 6 months and 12 months, there were no significant differences in the ratio of myocardium perfusion defect among the three groups. Nonetheless the ratio of myocardium perfusion defect was significantly decreased at 6 and 12 months in the normoxia group. Similarly, there was a significant decrease in the ratio of myocardium perfusion defect in the hypoxia-preconditioned group. However, there was no significant decrease in the ratio of myocardium perfusion defect at 6 and 12 months in the control group.

4. Discussion

To our knowledge, CHINA-AMI is the first-in-man trial to determine the safety and feasibility of intracoronary administration of hypoxia-preconditioned BMCs for treatment of acute ST segment elevation MI. The results of this study demonstrate that administration of hypoxia-preconditioned BMCs is comparable to that of normoxia BMCs in

Table 3
Safety summary by 1 month and 12 month follow-ups.

Safety summary	Normoxia (n = 10)	Hypoxia (n = 11)	Control (n = 14)	P[a]
Before hospital discharge	No. of patients			
Death	0	0	0	/
Myocardial infarction	0	0	0	/
Sustained ventricular tachycardia	0	0	0	/
Stroke	0	0	0	/
Revascularization	0	0	0	/
1 month follow-up				
Death	0	1 (9.1)	2 (14.3)	0.76
Myocardial infarction	0	0	0	/
Sustained ventricular tachycardia	0	0	0	/
Stroke	0	0	0	/
Rehospitalization for heart failure	0	0	0	/
Revascularization	0	0	0	/
Target-vessel revascularization	0	0	0	/
Stent thrombosis	0	0	0	/
Non-target-vessel revascularization	0	0	0	/
1 year follow-up				
Death	0	0	0	/
Myocardial infarction	0	0	0	/
Sustained Ventricular tachycardia	0	0	0	/
Stroke	0	0	0	/
Rehospitalization for heart failure	3 (30.0)	1 (9.1)	0	0.055
Revascularization	0	0	0	/
Target-vessel revascularization	0	1 (9.1)	0	0.61
Stent thrombosis	0	0	0	/
Non-target-vessel revascularization	0	0	0	/
Total no. of SAEs	3 (30.0)	2 (18.2)	2 (14.3)	0.77
Total no. of AEs	5 (50.0)	4 (36.4)	6 (42.9)	0.91

[a] Fisher exact test.

terms of safety and feasibility. We observed no significant major adverse effect in terms of MACEs or proarrhythmia following administration of hypoxia-preconditioned or normoxia BMCs. In both cell treated groups, there was significant improvement in myocardial perfusion following administration of hypoxia-preconditioned or normoxia BMCs as determined by SPECT. Compared with the normoxia group or control, there was significant improvement of LVDEV and LVESV at 6 and 12 months in hypoxia preconditioned group. But there was no significant improvement in LVEF after administration of hypoxia-preconditioned BMCs. Nevertheless, this trial was not designed to compare the clinical efficacy of hypoxia-preconditioned versus normoxia BMCs but to confirm the safety of hypoxia-preconditioned BMCs.

Table 4
Echocardiographic parameters.

Variables	Normoxia	Hypoxia-preconditioned	Control	P value
LVEF				
Baseline	56.9 (12.4)	50.9 (12.9)	57.1 (11.6)	0.39
6 months	59.1 (7.0)	53.2 (14.2)	55.8 (8.4)	0.20
Absolute difference	0.4 (7.4)	1.6 (6.4)	−4.9 (7.1)*	0.08
12 months	56.8 (9.6)	56.1 (11.1)	59.6 (5.2)	0.73
Absolute difference	0.6 (7.2)	3.9 (6.5)	−3.0 (8.9)	0.21
LVEDV				
Baseline	115.2 (42.5)	136.9 (34.7)	109.2 (19.7)	0.14
6 months	96.4 (22.5)	104.2 (33.5)	105.7 (30.5)	0.13
Absolute difference	−16.2 (31.6)	−28.8 (37.9)	9.1 (17.3)	0.03
12 months	120.2 (49.9)	101.5 (24.6)	102.7 (47.0)	0.10
Absolute difference	6.9 (22.62)	−30.5 (33.74)*	17.8 (43.5)	0.04
LVESV				
Baseline	55.1 (32.2)	72.8 (32.8)	48.8 (21.8)	0.16
6 months	40.3 (15.2)	54.0 (31.0)	48.0 (18.2)	0.04
Absolute difference	−11.1 (20.8)	−13.4 (16.1)*	10.6 (14.4)*	0.01
12 months	49.9 (34.4)	45.8 (18.1)	45.4 (25.8)	0.10
Absolute difference	−6.8 (13.7)	−23.6 (24.2)*	16.8 (26.7)	0.01
WMSI				
Baseline	1.3 (0.5)	1.5 (0.4)	1.4 (0.3)	0.69
6 months	1.1 (0.2)	1.2 (0.3)	1.2 (0.1)	0.97
Absolute difference	−0.1 (0.1)	−0.2 (0.2)*	−0.1 (0.2)	0.57
12 months	1.2 (0.3)	1.2 (0.3)	1.1 (0.1)	0.47
Absolute difference	−0.1 (0.1)*	−0.2 (0.2)*	−0.2 (0.3)	0.33

LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; WMSI, wall motion score index.
Data are means (SD) unless otherwise stated.
* $P < 0.05$ versus baseline within the same group.

Although BMC therapy has been a promising approach for MI treatment, poor therapeutic efficacy still is the major issue.[1,2,18,19] A Cochrane meta-analysis suggested mild improvement in left ventricular function measured by MRI.[20] So efficacy optimization is the challenge of stem cell therapy for MI. Prior experimental studies [11,21,22] have shown that modification of BMCs, using gene transfer, drug pretreatment and hypoxia preconditioning can enhance stem cell survival and improve their therapeutic efficiency. Compared with gene modification, hypoxia preconditioning of BMCs can be easily achieved by hypoxic culture and the potential risk associated with use of gene vectors is avoided [11,12]. Our previous pre-clinical studies [11] have demonstrated that hypoxia preconditioning can improve the therapeutic effect of BMCs after MI in rats. Hypoxia preconditioning of BMCs increases their expression of pro-survival and proangiogenic factors including hypoxia-inducible factor 1, angiopoietin-1, vascular endothelial growth factor and its receptor, Flk-1, erythropoietin, Bcl-2, and Bcl-xL [11]. These cytokines can further enhance the survival and therapeutic effects of BMCs.

The safety evaluation of hypoxia preconditioning is the first step to forward this approach to clinical application. Thus, we choose safety as the primary endpoint and it was evaluated by occurrence of major adverse cardiovascular events in this study. It is reported that there was no significant difference between the relative incidences of adverse events comparing BMCs and control groups at three years [2]. Meta-analysis also showed BMC therapy showed no difference with regards to mortality events when compared to placebo [23]. Here we found hypoxia-preconditioned BMC administration delivered by intracoronary infusion in acute myocardial infarction patients also demonstrated safety with one year follow-up. The adverse events of hypoxia-preconditioned BMC therapy were similar with normoxia or control group. These results provide the basis for further large size study of this hypoxia preconditioning approach.

In this study, SPECT revealed that both normoxia and hypoxia-preconditioned BMCs significantly improved myocardial perfusion as determined by the reduced ratio of myocardium perfusion defect compared with baseline. Nevertheless, there was significant improvement of LVEDV and LVESV change in hypoxia group compared with normoxia and control groups. These findings suggest that although both types of BMCs can enhance myocardial perfusion following MI, hypoxia-preconditioned BMCs seem to be more effective in amelioration of adverse LV remodeling after MI. Nonetheless the trial was not designed to compare the clinical efficacy of hypoxia-preconditioned versus normoxia BMCs. Moreover, BMC therapy appears to be more effective in patients with severely impaired LV function after MI. In this study, patients recruited into the trial had only moderately reduced LVEF. This might account for the modest improvement in LVEF observed in this study. Therefore, further studies should be targeted to those patients with severely impaired LVEF. More importantly, the clinical efficacy of hypoxia-preconditioned BMCs will be addressed in the future phase II–III prospective randomized clinical trial in a larger cohort of patients.

5. Limitations

There are limitations to this study. First, the number of patients recruited in this pilot trial was small. Our results can only provide some initial assessment of the safety and feasibility of hypoxia-preconditioned BMCs. Second, the function of hypoxia-preconditioned and normoxia BMCs was not investigated.

Table 5
Quantitative of myocardium perfusion defect score by SPECT.

Variables	Normoxia	Hypoxia-preconditioned	Control	P value
Ratio of myocardium perfusion defect				
Baseline	26.9 (9.1)	27.3 (14.5)	27.9 (7.0)	0.98
6 months	20.8 (9.4)	22.4 (13.9)	26.2 (8.4)	0.10
Absolute difference	−6.1 (4.1)*	−4.9 (5.6)*	−1.7 (3.7)	0.10
12 months	21.3 (8.0)	20.4 (12.4)	25.0 (10.7)	0.49
Absolute difference	−5.9 (4.3)*	−3.4 (6.4)	−2.6 (5.0)	0.46

Data are means (SD) unless otherwise stated.
* $P < 0.05$ versus baseline within the same group.

6. Conclusion

In conclusion, our results provide the first-in-man evidence that intracoronary administration of hypoxia-preconditioned autologous BMCs following acute MI appears to be safe and feasible. These results provide the basis for future prospective randomized clinical trials in a larger patient cohort.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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