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A new hope of CD133+ bone marrow stem cell for functional exercise capacity improvement in low ejection fraction coronary artery bypass graft patients: a clinical trial

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ABSTRACT

Background: Patients with low ejection fraction who are undergoing coronary artery bypass graft (CABG) only have an insignificant improvement in ejection fraction. This condition will make a little hope to achieve an improvement in physical performance. But now, from a view study, CD133+ stem cells offer new hope for this situation. This study evaluates CD133+ bone marrow stem cells' role for functional exercise capacity improvement in low ejection fraction coronary artery bypass graft patients.

Methods: Thirty patients with ischemic heart disease who had ejection fraction <35% at the National Cardiovascular Center were randomized into 2 groups. The treatment group undergoes the CABG + CD133+ procedure and the control group undergoes the CABG only. All research subjects underwent follow-up before and 6 months after the procedure. Fraction ejection, scar size, wall motion score index, ventricular dimensions, myocardial perfusion measured by cardiac MRI, 6 Minutes Walking Test (6MWT) and Minnesota Living with Heart Failure Questionnaire (MLHFQ) as an additional parameter for physical performance and quality of life. Data was analyzed using SPSS version 21 for Windows.

Results: The results of the fraction ejection parameters showed a significant improvement in the treatment group, from 25.88±5.66% to 34.57%±11.31% compared to CABG only 30.18±3.85% to 31.61±7.89% (p=0.040), in the perfusion defect showed improvement but not significant, left ventricular end-systolic volume and left ventricular end-diastolic volume showed improvement with no significant result, scar size was found to be an improvement in the treatment group 10 persons (76.92%) compared to the control group 5 persons (38, 46%) (p=0.040), the wall motion score index and 6MWT showed a significant improvement in the treatment group (p=0.003 and p=0.03, respectively). The MLHFQ parameter showed improvement but not significant.

Conclusion: CD 133+ stem cell implantation in patients with low ejection fraction who undergo CABG provides improved myocardial function and indirectly improves functional exercise capacity and patients' quality of life.

Keywords: CABG, CD133+ Stem cell, Functional exercise capacity.
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INTRODUCTION

Ischemic heart failure patients with low ejection fraction (EF) treated with coronary artery bypass graft (CABG) only show an insignificant EF postoperatively increment. A study by Ilirjana et al. showed that the average EF postoperative was 25.6 ± 5.2 to 31.08 ± 0.55.[1]

A recent study shows a promising role of stem cell therapy in myocardial regeneration. The type of stem cell often used in those studies was bone marrow stem cell (BMSC) because it is easily obtained and has minimal side effects. BMSC is one type of adult stem cell that consists of mesenchymal stem cells (MSC), hematopoietic stem cells (HSC), and endothelial progenitor cells (EPC). From all of those types, HSC and EPC have proven to be effective in angiogenesis. Both HSC and EPC express CD 133+ on their surface; therefore, CD 133+ could be shown as both cells' biomarker.[2]

CD133+ stem cells release vascular endothelial growth factor (VEGF) and other growth hormones through paracrine effects that are useful in angiogenesis, apoptotic inhibition, activation of myocardial hibernating, and repair of the extracellular matrix. CD133+ cell implantation trials in CABG patients with low EF have been widely carried out. Stamm et al. injected CD133+ BMSC and found a significant improvement in myocardial perfusion followed by the

increase of EF in the treatment group at the sixth and 18th months.[3]

Therefore, this study aims to prove that CD133+ BMSC implantation in CABG patients with low EF can improve myocardial function followed by physical performance increment and better quality of life.

METHODS

This study was conducted from March 2016 to June 2018. Thirty ischemic heart disease patients with EF <35% at National Cardiovascular Center were randomized into two groups. Fifteen patients in the treatment group undergo a CABG + CD133+ procedure, and fifteen patients in the control group undergo CABG procedures only. All research subjects undergo follow-up before and six months after the procedure.

The inclusion criteria were patients with three-vessel Coronary Heart Disease (CHD) indicated for CABG with EF <35% and had agreed to participate in the study. In comparison, the patient's exclusion criteria were concomitant valve surgery, history of myocardial infarction <14 days, had contraindications for MRI, history of ventricular arrhythmias (≥Lown III), CKMB levels > 25 U / L, troponin I levels > 0.02 µm / L, coagulation disorders; including familial hemophilia, COPD patients, HIV (+), hepatitis B (+), HCV (+), ALT / AST levels > 1.5 times the upper normal value, creatinine> 2 g / dL, direct serum bilirubin> 3,0 mg / L, ventricular conduction/pacing disorders, electrolyte and blood gas analysis (BGA) disturbances, and who have received immunosuppressive therapy or cytotoxic agents or radiotherapy within 4 weeks before surgery. Then the study's drop-out criteria were patients who died after the CABG, patients who refused to follow up, and the length of the aortic cross clamp> 120 minutes or CPB duration> 180 minutes.

INTERVENTION PATHWAY

Patients were asked to come two days before the CABG procedure to the hospital. One day before the procedure, subjects in treatment groups who underwent CABG carried out bone marrow aspiration.

After bone marrow aspiration, CD133+ cell purification is carried out. Bone marrow aspirates are filtered and flowed into new blood bags to eliminate debris, then 12,5 ml aspirates taken for sterility testing and cell analysis using flow cytometry. The bone marrow aspirate in a 450 mL blood bag was added with PBS / EDTA / 0.5.5% HSA (phosphate buffer saline - EDTA, with 5% Human serum albumin) then been centrifuged. The supernatant was removed, then the suspension was added 1.5 mL Magnetic microbeads - anti CD133 labeling. The suspension was incubated for 30 minutes before the CD133+ cells were separated using the CliniMACS® Magnetic Separation Device (Miltenyi Biotec).

After CD133+ stem cells have been prepared, the patients underwent CABG surgery. After the end of final distal anastomosis, CD133+ stem cell implantation was performed in the hypoperfusion area (known by MRI examination), using a 1 mL syringe with a 25 G (0.5 x 25 mm) needle. After 40 times total injections, the aortic cross-clamp was removed, and the surgery continues as usual.

MEASUREMENTS

Assessment parameters are EF, scar size, wall motion score index, ventricular dimensions, myocardial perfusion, 6 Minutes Walking Test (6MWT), and Minnesota Living with Heart Failure Questionnaire (MLHFQ). All patients undergo cardiac MRI examinations before and six months after surgery using 1.5 T MRI. The parameters measured were perfusion defects, ejection fraction, ventricular dimensions, scar size, wall motion abnormalities. In contrast, examination for VEGF measurement uses Enzyme-linked Immunosorbent Assay (ELISA).

Six minutes walking test (6MWT) and MLHFQ are the parameters for the patients' physical performance and quality of life. In the 6MWT, patients are asked to walk for 6 minutes on a ± 30-meter-long track. During the procedure, the patient can slow down, stop, or rest if necessary. 6MWT results were reported in meters. MLHFQ consisted of 21 questions, each of which had a value between 0 and 5. A value of 0 if there were no symptoms at all, and a value of 5 if the symptoms were very real and disturbing. The patients were asked all the questions simultaneously. The value of each question will be totaled.

STATISTICAL ANALYSIS

All statistical data were analyzed using IBM SPSS Statistics version 21.0 (SPSS Inc, Chicago, IL, USA) for Windows. Continuous data are presented as means ± standard deviation (SD) or median and interquartile range. Categorical data are presented as numbers (n) and percentages. The Shapiro-Wilk test is used to assess normality. Data were analyzed by an independent T-test or Mann-Whitney test. The comparative hypothesis test data is displayed in tabular form. The results of statistical analysis are significant if the p-value <0.05.

RESULTS

Two patients in both groups were drop-out of the research, resulting in only 26 people participating; 13 in the control group and 13 in the treatment group. Baseline characteristics in both groups were similar (p>0.050), except for left ventricular ejection fraction (LVEF) (p=0.040). The LVEF baseline in the treatment group was lower (25.88±5.66%) than the control group (30.18±3.85%) (Table 1).

In the myocardial function assessment, there is a significant improvement in LVEF in the treatment group compared to the control group (Δ score: 8.69±9.49 vs. 1.43±7.87; p=0.040) (Table 2). We found a significant improvement in scar size in the treatment group compared to the control group (p=0.040). The Wall Motion Score Index (WMSI) also showed a significant improvement in the treatment group compared to the control group (Δ score 0.51±0.48 vs. -0.01±0.21; p=0.003) (Table 2). Left Ventricular End Systolic Volume (LVESV) and Left Ventricular End Diastolic Volume (LVEDV) showed improvement without significant statistical (ΔLVESV score: -11,04±74,75 vs -8,28±32,21; p>0,05) (ΔLVEDV score: -27.59±84.48 vs. -19.08±36.79; p> 0.050) (Table 2). Perfusion defect also showed improvement but was not statistically significant compared to the control group (p=0.320) (Table 2).

Table 1. Baseline characteristic of respondents

Variable	Groups (N=26)		p
	CD133+ (n=13)	Control (n=13)	
Age (Years) (mean±SD)	54.61±8.07	57.46±6.33	0.320
The male population, n (%)	12 (92.30)	12 (92.30)	1.000
Systolic blood pressure (mmHg) (mean±SD)	121.92±14.37	126.53±19.60	0.500
Diastolic blood pressure (mmHg) (mean±SD)	78.00±12.03	72.46±15.36	0.200
Risk factors, n (%)			
Smoking	11 (84.61)	9 (69.23)	0.650
Dyslipidemia	6 (46.15)	11 (84.61)	0.090
Hypertension	9 (69.23)	7 (53.84)	0.680
Family history	7 (53.84)	9 (69.23)	0.690
Menopause	1 (7.69)	1 (7.69)	1.000
Diabetes Mellitus	5 (38.46)	9 (69.23)	0.230
History of previous infarction	11 (84.61)	11 (84.61)	1.000
Blood glucose (mg/dL) (mean±SD)	128.38±35.74	129.00±35.21	0.690
NYHA Grade III-IV, n (%)	4 (30.76)	4 (30.76)	1.000
CCS Grade II-III, n (%)	1 (7.69)	2 (15.38)	0.530
6-MWT (m) (mean±SD)	297.07±72.56	308.92±79.37	0.530
LVEF (%) (mean±SD)	25.88±5.66	30.18±3.85	0.040*
Scar size (%) (mean±SD)	27.76±15.76	24.45±13.73	0.470
WMSI (mean±SD)	2.32±0.17	2.07±0.31	0.080
MLHFQ score (mean±SD)	30.30±13.73	21.46±8.76	0.530

MLHFQ: Minnesota Living with Heart Failure Questionnaire; CCS: Canadian Cardiovascular Society; NYHA: New York Heart Association; LVEF: Left Ventricular Ejection Fraction; WMSI: Wall Motion Score Index; 6-MWT: 6 Minutes Walking Test; *Statistically significant if p-value less than 0.050.

6-MWT and MLHFQ evaluated physical performance and quality of life parameters. 6-MWT showed a significant improvement in the treatment group (Δ Score: 113.15±97.47 vs. 58.84±42.69; p=0.030) while the MLHFQ result was not significant (Δ Score: 18.38±17.89 vs. 9.00±7.30; p=0.090) (Table 2).

DISCUSSION

In the last decade, stem cell therapy for myocardial regeneration continued to progress. Recent studies found that stem cells, endothelial cells, and cardiomyocytes in ischemic conditions can produce extracellular vesicles consisting of exosomes and microvesicle bodies. Both molecules have important non-coding genetic material called mi-RNA (micro-RNA). The mi-RNA has a role in cell-to-cell communication, gene silencing, and the ability to activate VEGF, useful for the myocardial regeneration process series.[4,5]

Those molecules activate several pathways; some of them are P13K / Akt and mTOR pathways. These pathways will induce HIF 1α (hypoxia-induced factory) to be dimerized with HIF 1β. Both will go to the targeted gene to produce growth factors (one of them is VEGF), cytokines, and extracellular matrix (ECM) protease, which will induce migration of CD 133+ bone marrow cells. Then, paracrine effects and autocrine induced cardiomyocyte, endothelial, stem cell residents, and CD 133+ bone marrow cells to activate neovascularization, cardiomyogenesis, and extracellular matrix production.[6]

The general process of myocardial regeneration explained above commonly occurs in hypoxic condition. CD133+ bone marrow cells that naturally participate in the process were limited because they were used in various regeneration and differentiation processes in other systems. Therefore, external supplementation of CD133+ bone marrow cells in the myocardial could attenuate the process of angiogenesis, cardiomyogenesis, and extracellular matrix production.[3]

CABG is one of the options to improve the hypoxic condition in the myocardium through revascularization. However, this process alone could not give much difference to a patient with low EF. Patients with low EF generally have hibernating myocardium and a wide area of infarcted cardiomyocytes, which could not be reversed with revascularization alone. Revascularization only affects the main coronary artery; this alone depends on the remaining cardiomyocyte, endothelial, and stem cell residents. Complete restoration of myocardium needs angiogenesis, neovascularization, cardiomyogenesis, and extracellular matrix production, which will be maximized if CABG was followed by trans epicardial implantation of autologous CD133+ bone marrow cells.[7]

This study shows significant improvement of myocardial function measured by scar size, WMSI, and LVEF. It is also accompanied by a significant increment in VEGF levels, except ventricular dimensions and perfusion defects variables. There is also a significant improvement in physical performance measured by 6MWT, but the MLHFQ parameter shows improvement without statistically significant. Therefore, we could conclude that improvement in cardiac function could improve physical performance and quality of life. These results were quite similar to the TAC-HFT trial by Heldman et al., which compared the safety of autologous MSCs in patients with ischaemic cardiomyopathy with that of bone marrow mononuclear cells (BMCs). Their secondary efficacy showed

Table 2. Ejection Fraction, LVESV, LVEDV, WMSI, VEGF, 6-MWT, and MLHFQ parameters before and after intervention

Variables	Group		p
	CABG+CD133+	CABG	
Ejection Fraction			
Before (pre-op)	25.88±5.66	30.18±3.85	
After (6 month follow up)	34.58±11.32	31.62±7.89	0.430
Δ score (p-value)	8.69±9.49 (p=0.060)	1.43±7.87 (p=0.524)	0.040*
LVESV			
Before (pre-op)	115.87±35.94	141.05±7.77	
After (6 month follow up)	126.91±86.03	149.33±44.96	0.070
Δ score (p-value)	-11.04±74.75 (p=0.650)	-8.28±32.21 (p=0.388)	>0.050
LVEDV			
Before (pre-op)	156.10±47.46	200.89±39.10	
After (6 month follow up)	183.68±94.99	219.97±35.64	0.060
Δ score (p-value)	-27.59±84.48 (p=0.600)	-19.08±36.79 (p=0.101)	>0.050
WMSI			
Before (pre-op)	2.32±0.17	2.07±0.31	
After (6 month follow up)	1.82±0.43	2.08±0.28	0.080
Δ score (p-value)	0.51±0.48 (p=0.003)	-0.01±0.21 (p=0.088)	0.003*
VEGF			
Before (pre-op)	46.86±141.20	40.46±40.08	
After (6 month follow up)	49.46 (11.98-90.92)	19.88±33.78	0.310
Δ score (p-value)	14.19±98.58 (p=0.203)	-20.58±31.50 (p=0.063)	0.010*
6-MWT			
Before (pre-op)	299 (260.5-350)	298 (255-371)	
After (6 month follow up)	420 (381-441)	378 (335-414.5)	0.100
Δ score (p-value)	113.15±97.47 (p=0.001)	58.84±42.69 (p<0.005)	0.030*
MLHFQ			
Before (pre-op)	27 (20-39.5)	23 (15-16)	
After (6 month follow up)	12 (4.5-16)	14 (4.5-15.5)	1.000
Δ score (p-value)	18.38 ± 17.89 (p=0.006)	9.00±7.30 (p=0.001)	0.090

MLHFQ: Minnesota Living with Heart Failure Questionnaire; LVESV: Left Ventricular End Systolic Volume; LVEDV: Left Ventricular End Diastolic Volume; VEGF: Vascular Endothelial Growth Factor; WMSI: Wall Motion Score Index; 6-MWT: 6 Minutes Walking Test; *Statistically significant if p-value less than 0.050.

a significant increase in MLHFQ and 6MWT in the treatment group compared with the placebo group.[8] Another trial called C-CURE shows that patients treated with stem cells at long-term follow-up for one year also showed improvement from the 6MWT examination.[9]

The improvement of EF in this study according to the formula, EF = stroke volume (SV) / end-diastolic volume (EDV). EF is directly proportional (linear) to SV; meanwhile, SV itself is directly proportional to cardiac output (CO) based on the formula CO = SV x heart rate (HR). CO is also directly proportional to VO2 (whole-body O2 consumption) based on formula VO2 = CO x (Cao2 -Cvo2). According to the description above, the increasement of

EF will improve CO so that cells' oxygen needs to carry out metabolism are achieved, which will improve physical performance.[10]

CONCLUSION

CD 133+ stem cell implantation in patients with low ejection fraction who undergo CABG provides improved myocardial function and indirectly improves patients' functional exercise capacity and quality of life.

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CONFLICT OF INTEREST

There is no competing interest regarding the manuscript.

ETHICS CONSIDERATION

This study has been approved by the Institutional Review Board and Ethics Committee of the National Cardiovascular Center and National University in the country, as well as registered in ClinicalTrial.gov (NCT028709330).

AUTHOR CONTRIBUTIONS

All authors equally contribute to the study from the conceptual framework, data acquisition, data analysis until reporting the study results through publication.

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