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Stem cell therapy combined with myocardial revascularization: a pilot study using the harvest technique

Abstract

Background: To assess the usefulness of intra-myocardial autologous stem cell injection as a hybrid procedure with coronary artery bypass grafting (CABG) compared to conventional CABG only in the patients with ischemic heart disease with non-viable or non-graftable myocardial segments.

Methods: After approval of Local Ethics Committee and obtaining written informed consent, a prospective, comparative randomized study was conducted on 30 ischemic heart patients with non-viable or non-graftable segments. They were divided into two groups. Group A (15 patients): where intra myocardial injection of autologous bone marrow mononuclear cells (ABM-MNCs) was done in conjunction with CABG. Group B (15 patients): where CABG was performed only. Resting and stress echocardiography follow up was done for 6 months.

Results: There was no mortality in either group. No complications were noted at a mean of 6 months after surgery. Group A showed a significant improvement (mean of 7.25% and P<0.001) in left ventricular ejection fraction (LVEF). Both end- diastolic (LVEDD) and end-systolic (LVESD) diameters showed significant decrease compared to pre- operative values. LVEDD decreased by a mean of 5.23 (P<0.001) while LVESD by a mean of4.21 (P<0.001).

Conclusion: Stem cell therapy combined with CABG improves cardiac function compared with revascularization alone. In the same time, it is safe, feasible, and reproducible and doesn't increase the risk of major adverse events or mortality.

Keywords: CABG, stem cells (cell transplantation), cardiac function

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Abbreviations: ABM-MNCs, autologous bone marrow mononuclear cells; CABG, coronary artery bypass grafting; CAC, Canadian angina classification; CMRI, cardiac magnetic resonance imaging; CI, confidence interval; DSE, dobutamine stress echocardiography; LVEF, left ventricular ejection fraction; EuroSCORE, European system for cardiac operative risk evaluation; IMR, ischemic mitral regurgitation; LVEDD, left ventricular enddiasolic diameter; LVESD, left ventricular endsystolic diameter; Maces, major adverse cardiac events; NYHA, New York heart association classification

Introduction

Diffuse coronary artery disease represents a large sector of ischemic heart patients in whom traditional modalities, percutaneous coronary interventions (PCI) or CABG, will not be efficient enough for revascularization and will fail to improve their quality of life. On the other hand, ischemic heart patients who were exposed to myocardial infarction have non-viable myocardial segments. Frequently, those segments are large enough to affect the global function of the heart. In this case, the condition ends by ischemic cardiomyopathy. Moreover, in those patients, traditional modalities will not be enough and will fail to improve the quality of life. Stem cell therapy may represent the hope for those types of patients.[1–12] Several studies discussed this promising therapy with different techniques and routes including surgical transepicardial (intramyocardial injection),[1,9–12] selective intracoronary artery infusion,[2–4] percutaneous transfemoral endoventricular (catheter-based transendocardial injection)[5,6] or retrograde coronary venous.[8] Here in, we aimed to assess the usefulness of intra-myocardial autologous stem cell injection as a hybrid procedure with CABG compared to conventional CABG only in those patients with ischemic heart disease with non-viable or non-graftable myocardial segments.

Methods

Design

This is a prospective, randomized double-armed (controlled) clinical trial.

Participants: Thirty ischemic heart patients who were already scheduled for first-time isolated CABG with either nonviable or ungraftable myocardial segments, which was not suitable for conventional forms of revascularization. There were 27 males and 3 females (their ages ranged between 44 to 74 years old with a mean of 58.77).

Primary endpoint: The primary efficacy endpoints were

a. A change in global LVEF at 6 months versus baseline which was used to calculate the intended sample size. An assumption: difference in mean LVEF = 3.5%; SD of 6-7%; drop out = 10%. For an independent sample t-test 30 paired resting and DSE per group are needed - including drop out are 30 per group; and

b. The proportion of patients with recovery of contraction in previously akinetic myocardial segments at month 6. The secondary efficacy end points were the change from baseline to month 6 in echocardiographic LV diameters, the functional

status, and quality of life assessed by the NYHA classification at the 6-month study point.

c. The safety endpoints were the 30-day and 6-month rates

d. Of major cardiac adverse events (MACEs), defined as the composite of cardiovascular- and non cardiovascular-related death, myocardial infarction, congestive heart failure, resuscitated sudden death, and stroke; and

e. Of ventricular dysrhythmias.

Intervention: Patients were divided into two groups. Group A (ABM-MNCs and CABG): included 15 patients (14 men, 57.1 ±7.0 years of age, range: 44-68 years) to whom CABG plus intramyocardial stem cell (autologous bone marrow mononuclear cells, ABM-MNCs) injection were done. Group B (CABG only): a control group that included 15 patients (13 men, 59.6±7.4 years of age, range: 45-74 years) who received the conventional CABG only. Follow up period was 6 months.

Prior to the beginning of the study, the following issues were fulfilled:

a. Approval of the Ethical Committee of Faculty of Medicine, Ain Shams University;

b. Administration approval about the study; and

c. Informed consent approved by the ethical committee and signed by every participant in the study.

d. Our target was the cardiac segments, not the heart as a whole. So, having non- contractile or ungraftable cardiac segments is the main issue in selecting our patients without limiting the ejection fraction below a certain figure, our patients might or might not have been of low cardiac functions. So, the inclusion criteria were:

e. Fit for surgery (compensated heart), patients who were in failure were excluded from this study;

f. Having one or more non-contractile cardiac segment proved by DSE, or having one or more ungraftable cardiac segment proved by coronary angiography; and

g. Signed informed consent. We excluded patients who were not fit for the surgery, recent (< 1month) myocardial infarction, having ventricular dysrhythmias, history of malignancy or those who refused to sign the informed consent.

Operative procedure: For group A, a bone marrow aspiration technique from the iliac crest and processing methods has been previously described.[12] The collected bone marrow (78±6ml) was immediately transferred to the hemapheresis unit for red blood cell depletion and volume reduction. Using this schedule, we targeted a total of 1×109ABM-MNCs while avoiding unnecessary red blood cell and platelet contamination and higher harvest volumes. Stem cell isolation was done using Harvest Technologies Kit (Plymouth, MA, USA) according to manufacturer protocol (www.harvesttech.com).[13]

After general anaesthesia, the patient was placed in the lateral position. Proper sterilization was done then 60 ml of bone marrow were aspirated from the iliac crest under complete aseptic conditions. Using a density gradient centrifugal BMAC 60-05system, (HARVEST, Boston, MA), bone marrow aspirate was separated into its components which included 15cc of concentrated mononuclear cells which contains both stromal and hematopoietic stem cells ready for direct intramyocardial injection. During the processing of cells, the patient was repositioned to the supine position to continue the CABG operation. After finishing all bypass-to-coronary-artery anastomoses, multiple intramyocardial injections of stem cell concentrate were done to all cardiac segments with more injections directed to the non-contractile or ungraftable cardiac segments (0.2ml per injection) using a 22-gauge hypodermic needle.[12] Identification of the areas that would be injected was done based on the results of the DSE and cardiac MRI. The heart was divided into 17 segments that represent possible sites for injection, as follows: anterior septum, posterior septum, anterior wall, Lateral wall, posterior wall and inferior wall. All are divided into: basal, middle and apical segments. For group B patients: only conventional CABG was done.

Data collection and main outcome measurements

Baseline evaluation data regarding patient demographic characteristics, NYHA and Canadian angina classification, symptoms, cardiac risk factors, comorbid conditions, EuroSCORE (6 or plus indicates a high risk), the number of viable and transmural segments, IMR, LVEF or LV dimensions by resting two dimensional echocardiography (17-segment LV model, 5 grades), DSE (at doses of 5, 10, 20 and 40μg kg-1min-1 at 3-min) and Cardiac Magnetic Resonance Imaging (CMRI); in the form of cine, Inversion recovery (delayed enhancement) and dynamic study (perfusion); selective coronary arteriography and contrast left ventriculography, were collected and analyzed prospectively. CMRI was performed only to group A as a pre-injection investigation to determine precisely the site of injection and facilitate injection from technical point of view.

The operative data (the surgical technique, number of bypass grafts performed per patient, mitral valve repair, ischemic time, bypass time, postoperative intra-aortic balloon pump use and medication at discharge) were collected and analyzed. Postoperative results and MACEs (death, myocardial infarction, coronary revascularization, and stroke) were collected and analyzed. Postoperative follow-up period was at least 6 months to be included in the study. Data were tabulated, revised, coded and was compared to the corresponding data 6 month after the surgery.

Statistical analysis

Group results were expressed as mean values (±SD). We compared the groups using Student t tests. Categorical measures were expressed as counts (%) and analyzed by the $\chi 2$ test. The level of significance for P values (2-sided) was set at 0.05. Statistical package for social sciences (SPSS 19 for Windows, SPSS Inc, Chicago, IL, USA) was used.

Results

Between 4 September 2009 and14 September 2011, 575 patients with triple vessel coronary artery disease were admitted to our department for isolated CABG. A total of 56 patients were included in the study, after consideration of inclusion and exclusion criteria. Twenty-six patients were excluded (11 were not meeting inclusion criteria, 14 declined to participate and one died between randomization and therapy). The final cohort of 30 patients included 15 patients in the ABM-MNC group and 15 controls. The baseline characteristics of the study group are reported in Table 1. The inclusion criteria in both groups were comparable. There were no clinically or statistically significant differences between the two groups with regard to age, gender, cardiac risk factors, symptoms, EuroSCORE, the number of viable and transmural segments, LVEF or LV dimensions. All patients presented with LV dysfunction and moderate to severe LV dilatation. All patients were in high (3-4) NYHA score and had cardiac risk factors (left main disease or equivalent in angiography, poor functions and a kinetic and non-viable segments in DSE). Seven patients (2 in group A and 5 in group B) showed significant IMR.

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Table 1 The baseline characteristics of patient population

Variables	Group A (n=15)	Group B (n=15)
Age years (mean ± SD)	57.1 ±7.0	59.6 ±7.4
Gender male, n (%)	14 (93)	13 (87)
Hypertension, n (%)	11 (73)	14 (93)
Diabetes mellitus, n (%)	9 (60)	9 (60)
Smoking, n (%)	10 (67)	8 (53)
Left main stem disease, n (%)	4 (27)	5 (33)
Akinesia, n (%)	11 (73)	10 (67)
IMR in DSE, n (%)	2 (13)	5 (33)
Viability in pre DSE, n (%)	7 (47)	5 (33)
NYHA class	3.22±0.44	3.20±0.41
CCS class	3.14 ±0.56	2.88±0.54
LVEF (%)	41.13±7.97	45.60±11.35
LVEF (%)(pre DSE)	42.63±7.41	43.10±5.94
LVEDD (mm)	60.56±1.94	61.15±4.79
LVESD(mm)	47.52±3.13	46.55 ±4.91

Variables expressed as mean ± SD

Multi-vessel disease was present in all patients. There were no differences between the two groups with regard to the surgical technique, number of bypass grafts performed per patient, ischemic time, postoperative intra-aortic balloon pump use and medication at discharge. As regards the characteristics of ABM-MNCs, the mean total bone marrow harvesting time was 27±5 min and the mean total procedural time for ABM-MNCs implantation was 13±3 min. The mean time between the harvest of bone marrow cells and injection was 122±17 min. Overall high-dose inotropic support (intravenous dopamine at >5μg kg−1 min−1, dobutamine, epinephrine) was used in 17 patients (56%) but no significant difference was detected between the two groups.

Main outcomes

There was neither operative nor early mortality in both groups. The clinical events during follow-up included one late (after 6 months) mortality in either group (combined events: death and stroke). The rate of occurrence of relevant major adverse cardiac events (MACEs) in 6-month follow-up period was found to be significant in group B compared to group A. Compared with control groups, group A showed a significant improvement of LVEF from baseline to follow-up (7.25±5.1%; 95% CI,1.36-9.44; P = 0.019) (Figure 1). The maximum LVEF change by DSE was 5.40% ±7.38; P = 0.001). Moreover, the overall change of LVEDD from baseline to follow-up favored group A (−5.23±2.5; 95% CI, 3.47−6.97; P = 0.0001) and the overall change of LVESD from baseline to follow-up favored also group A (−4.21± 6.1; 95% CI, 2.13−6.09; P = 0.001). NYHA score showed a highly significant improvement in group A patients (−1.3±0.71; P = 0.001).

In group A, preoperative CMRI showed LVEF ranging from 25-46% (mean 34.2±8.4%). Myocardial thickening in infarcted territory (percentage of scarring to the whole thickness) ranged from 25% to transmural scarring. Postoperative CMRI showed improved mean LVEF (from 34.2±8.4% to 41±10.23%). Concerning the percentage of scarring, the mean percentage improved from 56.42±3.35% to 51.44±6.86% (P=0.67 versus baseline) and decrease in scar size (P=0.59 versus baseline).

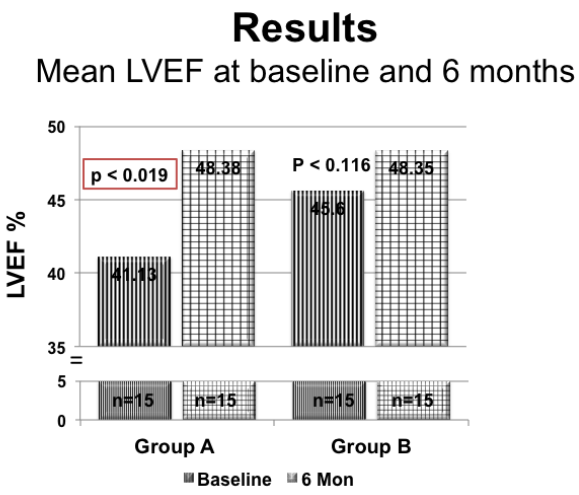

Figure 1 Mean LVEF at baseline and 6 months.

Major adverse cardiovascular events, including ventricular dysrhythmias and the composite of other cardiovascular events, were not significantly different between the two groups (relative risk for ventricular dysrhythmias (0.83; 95% CI, 0.39–2.32; P = 0.91; relative risk for cardiovascular event (1.13; 95%CI, 0.28–4.6; P = 0.86).

Two patients in group A had severe IMR; one had his mitral valve repaired during CABG together with stem cell injection. This patient died after 7 months (combined events: death and stroke). The other patient had no significant IMR in postoperative DSE. Five patients in group B had severe IMR; three of them had their mitral valve repaired by restrictive mitral annuloplasty. Post-operative DSE showed no significant IMR except one. The other two patients had moderate to moderately severe IMR together with 6 more patients who developed significant IMR in their post-operative DSE. So, the improvement was not significant in group B.

On comparison of the mean number of lesions and grafts done during revascularization, the numbers were very comparable in both groups. The post-operative viability in DSE in patients who had CABG with 3 grafts or more showed no significant improvement in both groups. Nevertheless, the correlation of induced-ischemia in high dose DSE in those patients was significant in group B and non-significant in group A.

In conclusion, group A, over group B, showed significant improvement in LVEF (both in resting and exercise echocardiography), LVEDD, LVESD and NYHA class. Further investigation in prospective randomized trials with long- term follow-up is warranted.

Discussion

Our study was done based on the results from preclinical studies showing that cellular therapy is a novel option to improve cardiac regeneration[14] or neovascularization.[15] Knowledge, as to the mechanisms of improvement in clinical trials, is inadequate. In chronic myocardial ischemia, human bone marrow may be the best-established source for adult stem cells, which consists of several subpopulations of pluripotent cells. Further advantages of these autologous cells are the avoidance of immunologic rejection, and their ready availability. Fresh methodology adopted in this study is based on implementation of ABM-MNCs aspirated from bone marrow directly to the ischemic myocardium after removal of erythrocytes; no time is required to

expand the cells in culture with this method while ABM-MNCs are directly delivered to the myocardial segments with multiple injections within approximately 3 hours. This is more convenient for patients as we don't have to bring the patient to the operating theatre to aspirate the bone marrow cells under general anesthesia and then 2-3 days later we get the patient back to the operating room for CABG. In addition, it is safer to avoid general anesthesia twice in such high risk patients. This procedure also helps to decrease the incidence of contamination of the sample during culture.

Angiogenic proteins released from ABM-MNC's (mostly CD34+ hematopoietic stem cells) improve angiogenesis and/or vasculogenesis at the ischemic myocardium; this is in keeping with the findings of many previously reported studies.[9] However, the use of unfractionated ABM-MNCs still raises many unanswered questions regarding the mechanisms involved and the dose–response effect, which are beyond the scope of this study. Furthermore, it has shown that ABM-MNCs isolated from those patients have a significantly reduced migratory and colony-forming activity in vitro and a reduced neovascularization capacity in vivo despite similar content of hematopoietic stem cells.[16] Different cellular implantation techniques have been performed including transepicardial.[1,9–12] selective intracoronary infusion[2–4] percutaneous transfemoral endoventricular[5,6] or coronary venous routes.[8] Each of these delivery methods has its distinct advantages and limitations and no single approach has gained favor as the optimal technique for cell transplantation. Surgical delivery of stem cells has been used, especially in models of chronic ischemic heart disease, and facilitates cell delivery to regions with occluded coronary vessels. Choosing the transepicardial intramyocardial implantation rather than intracoronary administration is supposed to overcome the problems of defective homing in functionally impaired stem cells. This route had been chosen aiming at the best results possible supported by the fact that the percentage of stem cell retained in the myocardium following intramyocardial injection is much higher (11±3%) compared with intracoronary route (2.6±0.3%) or retrograde coronary venous route (3.2±1.0%).[17,18]

CMRI has been used in our patients, only group A, as a widely accepted reference standard for the non-invasive assessment of myocardial ischemia and viability. Multiple studies have demonstrated that cine MRI is treated as a gold standard in imaging cardiac morphology, function and viability.[18] Furthermore, It was done as a pre-injection investigation to determine precisely the site of injection and facilitate injection from technical point of view.

Our preliminary results confirm those of previous studies[12] showing the safety and feasibility of direct intramyocardial injection of ABBMCs which did not lead to myocardial damage or severe dysrhythmia. In fact, significant increase in the rate of occurrence of relevant post-operative adverse events in group B patients including post-operative bleeding, dysrhythmia, chest pain, pneumothorax with surgical emphysema…etc. No intra-operative nor perioperative cardiac deaths. Postoperatively, one mortality occurred with no apparent cardiac cause. Clearly, our most interesting finding was the unexpectedly low mortality when compared to the expected rate described for these patients. The expected yearly mortality rate in patients with NYHA functional class III or higher, ranges between 20 and 30%;[11] in our study, 15 out of 15 patients in group A finished the 6-month follow up period of the study.

The results of our study showed marked clinical improvement related to stem cell therapy in the injected patients. These results confirms those of many previous trials including Arom et al.[10] who reported improvement in NYHA class from preoperative of 2.9±0.7 to postoperative of 2.0±0.9 ($P<0.001$).[10] Group A patients showed significant improvement in LVEF in post- operative echocardiography and DSE compared to non-significant improvement in group B patients. These data also show the same results retrieved from previous studies worldwide which confirm the potential improvement in LVEF in patients who received stem cell therapy during CABG. This improvement in LVEF in group A is explained by the improvement in cardiac dimensions postoperatively. Both LVEDD and LVESD showed significant decrease compared to preoperative values which indicates a decrease in myocardial remodeling postoperatively. Group B patients showed no significant improvement in both parameters. Comparing our results with those of some of the most widely accepted studies that used stem cell therapy with CABG [1,9-12], we will find that our results are very comparable.

Limitation of the study

The major limitation of this study is the sample size (both the number of patients enrolled and the number of clinical events) was small, which limits the power of the statistical analyses and the strength of the subsequent conclusions about efficacy. The control group was not assigned concurrently with treated patients and did not receive placebo injections because of Ethics Committee concerns. It is possible that this inadvertent selection bias influenced the study results. Because of financial causes, lack of CMRI for the control group and lack of positron emission tomography investigation for both groups, for the measurement of perfusion, which is considered the gold standard for assessment of myocardial viability in patients with ischemic cardiomyopathy, should be mentioned as another limitation for this study. Lastly, the follow-up is still limited to 6 months.

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None.

Conflicts of interest

The authors declare there is no conflict of interests.

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