Evaluation of the autologous bone marrow mononuclear therapy and functional restoration in the scarred myocardium by imaging analysis

Alla Gopala Krishna Gokhale, Lakshmi Kiran Chelluri[1], K. Kumaresan[2], G. Subramanyam[3], K. Sudhakar[3], Sathish Vemuri[1], Tanya Debnath[1], K. S. Ratnakar[4]

Department of Cardiothoracic Surgery, [1]Department of Transplant Biology and Stem Cell Lab, [3]Department of Anesthesiology, [4]Department of Pathology, Global Hospitals, Lakdi-ka-Pool, [2]Department of Nuclear Medicine, KK Nuclear Scans Clinic, Somajiguda, Hyderabad -500 004, India.

Address for correspondence: *Dr. Lakshmi Kiran Chelluri, Consultant and Head, Department of Transplant Biology and Stem Cell Lab, Global Hospitals, Lakdi-ka-Pool, Hyderabad 500 004, Andhra Pradesh, India. E-mail: apparusu@hotmail.com*

ABSTRACT

A 62-year-old male patient with previous history of myocardial infarction, akinetic myocardial segments, and an ejection fraction of 31% with the NYHA class III category was selected for the autologous bone marrow (ABM)-derived mononuclear cell fraction injection during CABG surgery. Nitrate augmented myocardial tracer uptake was imaged by ECG gated SPECT pre- and 1 year post-ABM therapy, using radiotracer Tc99m Sestamibi. The baseline gated SPECT demonstrated full thickness infarct in 40% area of LAD territory. Bone marrow aspirate of 20.0 ml from sternum yielding a mono nuclear cell fraction of 4.5×10^7 cells/ml was suspended in 2.0 ml of sterile normal saline to be injected at eight sites of the injured myocardium. There were no apparent side effects due to the procedure, i.e., life threatening events, major bleeds, reaction, or shock. The case was followed at the end of 1, 3, 6 months by ECG and Holter monitor and ECG gated SPECT at the end of 12 months. The gated SPECT images demonstrated mild but definitely improved tracer uptake within part of the infarcted segments along with improvement in ejection fraction to 45%, and a clinical change in the NYHA Class to II. Cell-based therapy may offer benefits of induction of normal tissue microenvironment.

Key words: Autologous bone marrow mononuclear therapy, cardiovascular disease research, myocardial infarction, molecular imaging

INTRODUCTION

Cardiac remodeling using adult stem cell therapy in clinical trials of post-myocardial infarction (MI) injury, as the therapeutic strategy during cardiac surgery is entering clinical practice.[1] The experimental and clinical models in various studies have yielded equivocal results. At present, this treatment is highly promising to become a standard treatment due to its effectiveness. The variables are confounding in these studies. It is yet to be determined the appropriate cell dosing, cell type, timing of the therapy, choice of cases, and the degree of stem cell approach for treating these otherwise nonamenable to therapeutic option cases.[2] Here we report adult stem cell therapy for myocardial revascularization and repair during CABG surgery. It is also important to determine objectively the functional improvement in these cases due to stem/progenitor cell therapy alone. Molecular imaging is rapidly taking the center stage in determining the effects of the stem cell therapy *in vivo* and the long-term follow-up.[3] Molecular imaging itself is currently evolving with focus

Access this article online

Quick Response Code:

Website: www.jcdronline.com

DOI: 10.4103/0975-3583.83037

on the multimodal approach for addressing the issues of stem cell homing, proliferation, and differentiation.[4] It has been, therefore, aimed at, to assess the improvement in the LV function and the global ejection fraction, using autologous bone marrow mononuclear fraction as an adjunct to CABG surgery, using noninvasive molecular imaging, 2D Echo along with the changes in the NYHA class changes in a case of scarred myocardium due to severe myocardial infarction.

MATERIALS AND METHODS

The study has been IRB/IEC approved. High risk video consent was obtained. The case has been recruited into the study which is registered with ICMR with universal trial number: TEMP UTRN 042233121-1506201022763720.

Screening procedures

The case was screened at the baseline for the extent of scarred myocardium using ECG gated SPECT imaging of myocardium after rest injection of Tc99m Sestamibi augmented with sublingual nitrate preparation. NYHA class was determined based on the questionnaire of NYHA and was classified under category III. The cardiac MRI and SPECT images were addressed at the baseline and the ejection fraction was estimated along with the 2D-echocardiogram. Akinetic and dyskinetic segments were assessed.

Bone marrow aspiration

Bone marrow (20.0 ml) from sternum during coronary bypass surgery (CABG) from 62-year-old male was aspirated under general anesthesia (GA).

Mononuclear fraction isolation

The bone marrow was equally diluted with normal saline, and the mononuclear fraction was isolated using the clinical grade Ficoll gradient and centrifugation method (Sigma, USA). The cells were counted in a coulter counter and viability was checked using the trypan blue dye exclusion method. The sample was assessed for CD34+/CD45+ cells (0.7%) using FACS Calibur prior to dispatch of cells to the theater before the completion of the CABG surgery. The cells thus isolated were suspended in 2.0 ml of normal saline.

Stem cell therapy

The cell suspension was injected circumferentially at eight sites of 0.1 ml volume of cell suspension each in this case, surrounding the scarred myocardium.

Follow-up

The case was followed up periodically at 1, 3, 6 months using ECG, Holter monitor and ECG gated SPECT at the end of 1 year. The parameters assessed included the global ejection fraction, increased perfusion using SPECT image analysis, end-diastolic volume (EDV), end-systolic volume

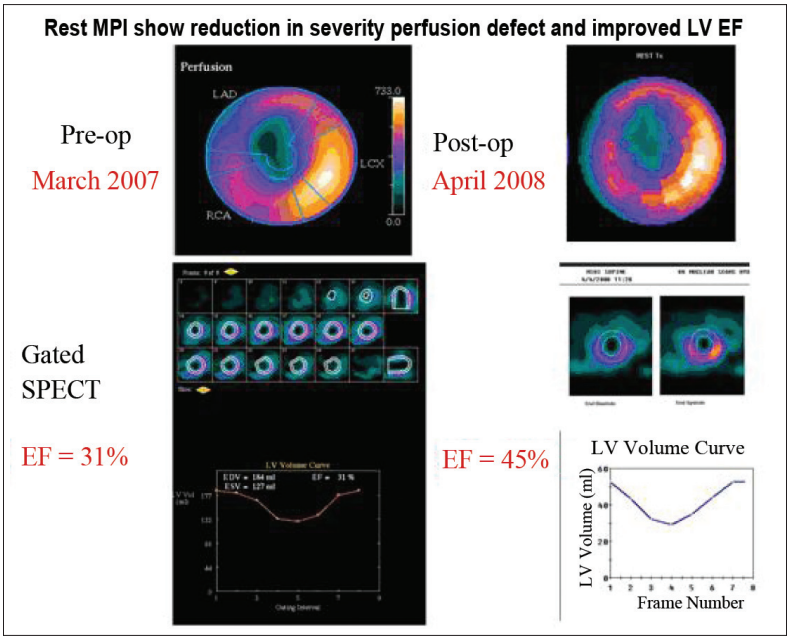

Figure 1: Nitrate augmented myocardial tracer uptake was imaged by ECG gated SPECT using radiotracer Tc99m Sestamibi which revealed full thickness infarct in 40% area of LAD territory. At the end of 1-year period, improved tracer uptake could be demonstrated by SPECT within part of the infracted segments along with improvement in ejection fraction to 45%.

(ESV), echocardiography and changes in the NYHA class category and angina status.

RESULTS

A 62-year-old male patient with previous history of myocardial infarction, akinetic myocardial segments and an ejection fraction of <31% with the NYHA class III category was selected for the autologous bone marrow-derived mononuclear cell fraction injection during CABG surgery. Myocardial SPECT demonstrated full thickness infarct in 40% area of LAD territory. Bone marrow aspirate (20.0 ml) from sternum has yielded a mono nuclear cell fraction of 4.50×10^7 cells/ml with a positive 0.7% of CD45+/CD34+ cells. The cells were suspended in 2.0 ml of sterile normal saline to be injected at eight sites of the injured myocardium. There were no apparent side effects due to the procedure, i.e., life threatening events, major bleeds, reaction or shock. The case was followed at the end of 1, 3, 6 and 12 months. At the end of 1 year period, improved tracer uptake could be demonstrated by SPECT within part of the infarcted segments along with improvement in ejection fraction to 45% and clinical change in the NYHA Class to II [Figure 1].

Ejection fraction improved from 31% to 45% as demonstrated by the % gated SPECT analysis of the hemodynamic data. LV end-diastolic volume per ml was 184 ml prior to the therapy followed by 208 ml post-1 year. Stroke volume index improved from 33 ml/m^2 to 50 ml/ m^2. Similarly, LV end-systolic volume was 127 ml prior and 120 ml post-therapy. The infarct region as severe perfusion defect measured as % area under LAD territory has shown an improvement from 40% to 30%. The NYHA class demonstrated changes from Class III to Class II [Table 1].

Table 1: Cardiac function analysis at 1 year follow-up

Parameter	Before cell therapy	One year after cell therapy
Hemodynamic data		
LV ejection fraction,% by gated SPECT	31	45
Stroke volume index, ml/m^2	33	50
Cardiac geometry		
LV end-diastolic volume, ml	184	208
LV end-systolic volume, ml	127	120
Infarct region as severe perfusion defect (i.e., <30% of maximal activity) measured as % area under LAD	40%	30%
NYHA classification	Class III	Class II

Hemodynamic parameters and cardiac geometry have demonstrated improvement in the global ejection fraction from 31% to 45% by the % gated SPECT analysis. The infarct region as severe perfusion defect measured as % area under LAD territory has shown an improvement from 40% to 30%. The NYHA class demonstrated changes from Class III to Class II.

DISCUSSION

The crucial issues that need consideration, while using stem cell therapy as a treatment option either as standalone therapy or synergistic in nature, are the cell type, cell number, therapeutic time window, route of delivery and critical evaluation of therapeutic benefit.[5-8] As reported by the Strauer *et al*., it is thought that the use of bone marrow mononuclear fraction would include the hematopoietic and nonhematopoietic cells that would be actively harnessing the tissue microenvironment to a desired protection level.[9] The preclinical models have demonstrated that hematopoietic fraction could differentiate to lineages that could regenerate the damaged tissue due to *in vivo* differentiation to cardiomyocytes using host niche (microenvironment).[10] The nonhematopoietic component owing to its immunomodulatory properties and release of trophic factors is assumed that it could induce the remodeling of the injured myocardium in a synergistic way to accelerate the repair process and regenerate a viable tissue and thereby increase the LV function.[11] There are preclinical reports suggesting the route of delivery as other important criteria for the accessibility of the stem cells. It was observed that the i.v. infusion of stem cells would be of little value as they would primarily go to liver, spleen and the residual fraction reaching the injured myocardium may not be adequate and hence, the controversial results of lack of improvement in some studies. other studies have demonstrated good results using combination of routes, i.e., intracoronary and intramyocardial for the maximum homing of cells for tissue repair.[12] However, other studies did not corroborate similar findings owing to complex variables and confounding factors. In order to maximize the availability of viable cell to the scarred tissue, direct small volume injections at number of sites surrounding the injured myocardium was administered to the case under discussion. Reports from Lunde and Aakhus indicated the long-term improvement in acute myocardial infarction (AMI) using intracoronary mBMC treatment.[13] The optimum cell dose for the stem treatment is yet to be determined. However, the dose that we adopted in this case was as per our previous publication, where the improvement of LV function was dose-dependent.[14] Meluzín *et al*. reported that mBMC transplants demonstrate regional myocardial function of the infarcted wall in a dose-dependent manner.[15] In an another study of dilated cardiomyopathy, our results corroborated with the group on the improved ejection fraction with increased CD34+/CD45+ cells.[15] Dose-dependent improvement is a significant observation from different groups necessitating a need to arrive at an algorithm based on variable factors. And a large

cohort of the sample size from all over world can alone circumvent the dose criteria. The current follow-up criteria from each study group thus far have singularly focused on the functional improvement. With the availability of noninvasive technology, it is now possible to address the structural stratification *vis-à-vis* the functional improvement.[16,17] The reported case has significantly demonstrated the increased perfusion and the improved viable myocardium on the rest MPI gated SPECT images due to stem cells although it may be argued that CABG is responsible for the LV function improvement. The gated SPECT images demonstrate the nitrate augmented myocardial tracer uptake using radiotracer Tc99m Sestamibi which revealed improved tracer uptake within part of the infarcted segments along with improvement in ejection fraction to 45%. It is pertinent to the stem cell therapy to optimize the therapeutic window time as this may limit the performance of the cells in the acute stage with inflammatory responses and the implications thereafter in the AMI.[18] With the advances in the imaging technology, it is important to address the stem cell tracking for its homing, proliferation, and differentiation potential to be able to provide tangible solution to the critical factors and the benefit of the stem cell therapy in modern medicine.

REFERENCES

1. Qiu B, Yang X. Molecular MRI of Hematopoietic Stem progenitor Cells: Molecular MRI of Stem-Progenitor Cells Homing to Atherosclerotic Plaques. Nat Clin Pract Cardiovasc Med 2008;5:396-404.
2. Bartunek J, Croissant JD, Wijns W, Gofflot S, de Lavareille A, Vanderheyden M, *et al*. Pretreatment of adult bone marrow mesenchymal stem cells with cardiomyogenic growth factors and repair of the chronically infarcted myocardium. Am J Physiol Heart Circ Physiol 2007;292:H1095-104.
3. Fratt L. Molecular Imaging Meets Cardiac Stem Cell Therapy. Mol Imaging 2009;3:1-4.
4. Nahrendorf M, Sosnovik DE, French BA, Swirski FK, Bengel F, Sadeghi MM, *et al*. Advances in Cardiovascular Imaging. Multimodality Cardiovascular Molecular Imaging, Part II. Circ Cardiovasc Imaging 2009;2:56-70.
5. Dawn B, Tiwari S, Kucia MJ, Zubasurma EK, Guo Y, Sanganalmath SK, *et al*. Transplantation of bone marrow derived very small embryonic like stem cells attenuates left ventricular dysfunctional and remodeling after myocardial infraction. Stem cells 2008;26:1646-55.
6. Piao H, Youn TJ, Kwon JS, Kim YH, Bae JW, Bora S, *et al*. Effects of bone marrow derived mesenchymal stem cells transplantation in acutely infracting myocardium. Eur J Heart Fail 2005;7:730-8.
7. Zhang S, Jia Z, Gej, Gong L, Ma Y, Li T, *et al*. Purified human bone marrow multipotent mesenchymal stem cells regenerate infarcted myocardium in experimental rats. Cell Transplant 2005;7:730-8.
8. Nagaya N, Fujji T, Iwase T, Ohgushi H, Itoh T, Uematsu M, *et al*. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis . AM J Physiol Heart Circ Physiol 2004;287:H2670-6.
9. Strauer BE, Ott G, Schannwell CM, Brehm M. Bone marrow cells to improve ventricular function. Heart 2009;95:98-9.
10. Gojo S, Gojo N, Takeda Y, Mori TM, Abe H, Kyop S, *et al*. *In vivo* cardiovasuculogenesis by direct injection of isolated adult mesenchymal stem cells. Exp Cell Res 2003;288:51-9.
11. Gersh BJ, Simari RD, Behfar A, Terzic CM, Terzic A. Cardiac Cell Repair Therapy: A Clinical Perspective. Mayo Clin Proc 2009;84:876-92.
12. Anderson CJ, Bulte JW, Chen K, Chen X, Khaw BA, Shokeen M, *et al*. Design of targetd Cardiovascular Molecular Imaging Probes. J Nucl Med 2010;51:3s-17s.
13. Lunde K, Aakhus S. Cell therapy in acute myocardial infarction: Measures of efficacy. Heart 2008;94:969-70.
14. Kaparthi PL, Gupta N, Chelluri LK, Adavi V, Rao VS, Ratnakar KS, *et al*. Autologous bone marrow mononuclear cell delivery to dilated cardiomyopathy patients: A clinical trial. Afr J Biotechnol 2008;7;207-10.
15. Meluzín J, Mayer J, Groch L, Janousek S, Hornácek I, Hlinomaz O, *et al*. Autologous transplantation of mono nuclear bone marrow cells in patients with acute myocardial infarction: The effect of the dose of transplanted cells on myocardial function. Am Heart J 2006;152:975.e9-15.
16. Beitnes JO, Hopp E, Lunde K, Solheim S, Arnesen H, Brinchmann JE, *et al*. Long term results after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction; The ASTAMI randomized, controlled study. Heart 2009;95;1983-9.
17. Feigenbaum GS, Lemberg L, Hare JM. Tracking cell fate with non-invasive imaging. J Am Coll Cardiol 2009;54:1627-8.
18. Xu S, Xu X, Guo Y, Gao W. Inflammatory responses after intracoronary mononuclear bone marrow cell therapy in swine. Bone Marrow Transplant 2009;44;427-31.

How to cite this article: Gokhale AK, Chelluri LK, Kumaresan K, Subramanyam G, Sudhakar K, Vemuri S, Debnath T, Ratnakar KS. Evaluation of the autologous bone marrow mononuclear therapy and functional restoration in the scarred myocardium by imaging analysis. J Cardiovasc Dis Res 2011;2:133-6.
Source of Support: Nil, **Conflict of Interest:** None declared.