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Full Length Research Paper

Impact of intracoronary autologous bone marrow mononuclear cells in patients with dilated cardiomyopathy

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The impact of intracoronary autologous bone marrow mononuclear cells (BMMC) in patients with DCM (dilated cardiomyopathy) with severe left ventricular (LV) dysfunction was assessed. The study included five DCM patients (age group 20 - 65 years) with ejection fraction (EF) of ≤ 30%. Mononuclear cells (Mean MNC count: 9.16 x 10^7 and Mean CD34 positive cell count: 3.68 x 10^5) were infused through intracoronary injection into the patients. Left ventricular ejection fraction (LVEF) was increased from 25 30% to 40 - 45% (approximately 500,000 CD34 + cells) in two of the patients. Conclusively, bone marrow mononuclear cell therapy has a potential to be considered a standard therapeutic entity for the treatment of dilated cardiomyopathy with severe LV dysfunction.

Key words: Mononuclear cells, dilated cardiomyopathy, autologous, intra-coronary.

INTRODUCTION

Patients suffering from several cardiomyopathies such as dilated cardiomyopathy (DCM) have increased world-wide, constituting a serious public health problem. DCM is caused by the impairment of left/or right ventricular systolic pump function, leading to progressive cardiac enlargement, a process called remodelling and often producing symptoms of congestive heart failure. Approximately 20% of patients have familial forms of disease with mutations of genes encoding myocardial structural proteins and transcription factors that control the expression of other myocyte genes (Maron et al., 2006). Prevalence rate of DCM is approximately 1 in 5,439 or 0.02% or 50,000 people in USA. Idiopathic DCM, a primary myocardial disease of unknown etiology characterized by a loss of cardiomyocytes with replacement by fibroblasts, is an important cause of heart failure. Thus, restoring lost myocardium would be desirable for the treatment of DCM (Nagaya et al., 2005). Heart failure Functional Class (FC) NYHA III and IV has been one of the main causes of hospital admissions, which utilize a large part of healthcare resources with a high mortality rate (40%/year). Despite the progresses made regarding the drug therapy of chronic systolic heart failure, for a significant number of individuals, the syndrome progresses relentlessly. Therefore, the development of new therapeutic procedures, such as the intracoronary or intramyocardial implant of stem cells (mononuclear and mesenchymal) derived from bone-marrow aspirate from the individual's own bone marrow (autologous), constitutes a promising therapeutic option for these advanced cases (Helena et al., 2006). For the homing of these cells, cytokine, such as stromal-cell-derived factor 1, activation, may be required which are already up-regulated following cardiomyopathy (CMP) (Maddury et al., 2006; Askari et al., 2003).

There is a huge deal of controversy in literature on the capacity of bone marrow mononuclear cells (BMMC) to regenerate cardiomyocytes. However, several clinical and experimental studies showed the capacity of bone marrow-derived cells to improve heart performance when injected directly into the myocardium or in the systemic circulation (Maddury et al., 2006; Strauer et al., 2002; Tse et al., 2003) in myocardial dysfunction.

The primary objective of this study is to assess the impact of intracoronary autologous BMMC in 5 patients

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Table 1. Inclusion/ Exclusion criteria for the patients in the study.

Inclusion criteria	Exclusion criteria
1. Exertional angina class III-IV 2. Severe LV systolic dysfunction with EF ≤ 30%	1. Severe Co-morbid medical condition 2. LVEF ≥ 31%
3. Single/Multi vessel coronary artery disease neither suitable for CABG nor PTCA + stenting	3. Stroke with significant sequel. 4. HIV/HBS/HCV Positive serology 5. Short life expectancy due to cancer/terminal illness
4. Hypokinetic, Akinetic, Dyskinetic segments	6. Mental Disorders 7. Gross CCF/Pulmonary edema

Table 2. Clinical details of the patients.

Patient	Age/sex	Clinical Hx
P1	65/M	Dilated/Ischaemic CMP/NYHA Class II Mild CAD
P2	70/M	Dilated CMP/NYHA Class III, IV/CAD
P3	23/F	CM with severe LV dysfunc NYHA Class II CAD
P4	60/M	DCM with severe LV dysfunc NYHA Class II CAD
P5	25/F	DCM with severe LV dysfunc

with dilated cardiomyopathy with severe left ventricular (LV) dysfunction, angina and mild coronary artery disease (CAD). We assessed the safety and efficacy of the procedure with no notified adverse events.

METHODS

This study is an Institutional Review Board (IRB) approved project which includes five patients who were treated for dilated cardiomyopathy with coronary artery disease (CAD) by cell therapy in the year 2006 at Global Hospital, Hyderabad. Inclusion/ Exclusion criteria for the study are as listed in Table 1. All the patients (age group 20 - 65 years) presented with a history of dilated cardiomyopathy with severe LV dysfunction [ejection fraction (EF) of ≤ 30%] and minimal coronary artery disease falling under either New York Heart Association (NYHA) Class II or III; Shortness of Breath (SOB); orthopnea and Paroxystic nocturnal dyspnea (PND).

They were followed with catheterization profile and haematological parameters for BM aspiration under GA. Breathlessness severity, LV ejection fraction, exercise capacity, and inducible ischaemia and arrythmias are validated at baseline, 1, 3 and 6 months follow up. Echo 16 segment analysis was considered as follow up parameter to monitor the patient prognosis post stem cell therapy.

Stem cell therapy

High risk informed consent was obtained from the patient and his/her relatives. BM aspiration was performed under aseptic precaution under general anaesthesia, from the right iliac crest. Mononuclear cells were isolated using Ficoll-gradient. Mononuclear cells were suspended in 45 ml of normal saline. 30 ml was injected in the left coronary artery and 15 ml in the right coronary artery slowly through Right Radial approach. The time taken for left coronary injection was 10 - 12 min while for right coronary injection was 5 - 6 min. Approximately 10 cc injection was given at one bolus.

BM mononuclear cell (MNC) count, viability and CD34 count (flow cytometry method) were done on every patient. Patient was discharged from the hospital after 72 h post-procedure. Follow up of the patient was done at 1st, 3rd and finally at 6th month (total: 3 visits). 1. Echo (Left ventricular ejection fraction) at 1, 3 and 6 months was taken as an improvement measure.2. Reduction in the requirement of drug therapy over a period of time post stem cell therapy was another parameter that was considered for follow up study.3. Improvement in NYHA functional class was observed at follow up.

RESULTS AND DISCUSSION

Clinical details of the patients studied are summarised in Table 2. Volume of bone marrow aspirated, mononuclear count, viability and CD34 positive count with the clinical outcome (LVEF before and after therapy) are summarised in Table 3. No occurrence of pain, arrhythmia or hemodynamic instability during the procedure and in the immediate post-procedure period was observed in any of the patients. Electrocardiographic (ECG) and enzymatic monitoring was carried out during the first 24 h post-procedure in an intensive care unit, with no alterations of these parameters. LV function was significantly improved after the therapy. LVEF increased from 25 - 30% to 40 - 45% after the MNC injection in two of the patients. Patients were clinically improved from NYHA class III to NYHA class I.

So far, in all animal models of cardiopathy and clinical assays, cell therapy (BMMNCs) promoted an improvement in cardiac function (Helena et al., 2006). In patients with chronic Chagas cardiopathy with severe systolic dysfunction, the intracoronary release of BMMC also showed to be safe and resulted in functional improve-

Table 3. Summary of the mononuclear count, viability and CD34 positive count with the clinical outcome (LVEF before and after therapy) of the patients.

Patient	Age/sex	MNC	Viability (%)	CD34 (%)	No. of CD34 infused	LVEF (%)	
						Before	After
P1	65/M	$1.4x10^{8}$	98	0.4	$5.6x10^{5}$	25	45
P2	70/M	$6.8x10^{7}$	94	0.37	$2.5x10^{5}$	30	40
P3	23/F	$6.7x10^{7}$	94	0.44	$2.9x10^{5}$	30	35
P4	60/M	$1.3x10^{7}$	96	0.4	$5.2x10^{5}$	25	40
P5	25/F	$1.7x10^{8}$	99	0.13	$2.2x10^{5}$	30	35

ment (Fabio Vilas et al., 2004; Milena et al., 2004). Based on these results, our preliminary study was aimed at evaluating the outcome of autologous BMMNC intracoronary injection into five patients suffering from dilated cardiomyopathy (NYHA class III) with severe LV systolic dysfunction with EF ≤ 30%. We observed that the procedure was carried out with no adverse events, suggesting the method is safe and viable. In addition, remarkable recovery (improvement in Echo parameters) was observed in two of these patients after the stem cell therapy. Both the patients were infused with approximately 500,000 CD34 positive cells. In patient 1 (P1), shown in Table 3, 5.6 x 105 CD34 positive cells with 98% viability were infused. To our interest, LVEF in this patient was appreciably raised from 25% before the therapy to 45% after the stem cell therapy, indicating the improved myocardial contractility and LV function. Similarly, Patient 4 (P4) was injected with 5.2 x105 CD34 positive cells with 96% viability. Again it was found that LVEF was reasonably increased from 25 to 40% post cell therapy. On the contrary, the rest of the patients who were provided with only approx 200,000 CD34 positive cells seemed to have improved LVEF percentage but this could not be deemed significant owing to a small rise. More data in terms of increase in number of patients are required in further studies to add more weight to our observation.

Importantly, we have restricted the treatment to patients with a LVEF of ≤30% following cardiomyopathy, thus enabling us to study patients with relatively large DCM-related myocardial damage. Concordant with the previous studies, we had chosen adult, mononuclear BMCs for injection as they are known to contain meso-dermal, hematopoietic, and endothelial progenitor cells. Thus, the regeneration of necrotic myocardium and vessels can be successfully achieved by several different fractions of mononuclear BMCs.

It is well known that intravenous application would require many circulation passages to enable infused cells to come into contact with the infarct related artery. Thus, supplying the entire complement of cells by intracoronary administration obviously seems to be advantageous because all cells are able to flow through the affected tissue during the immediate first passage (Strauer et al., 2002; Wollert and Drexler, 2006).

Very few clinical trials have been reported until today showing the marked improvement in myocardial function in patients with dilated cardiomyopathy (Bernardo et al., 2007). Strauer et al. (2002) demonstrated for the first time that transplanted intracoronary, autologous BMCs may lead to repair of infarcted tissue when applied during the immediate post infarction period (Maddury et al., 2006; Strauer et al., 2002). The TOPCARE study demonstrated a significant increase in global LVEF and a significant decrease in infarct size after the intracoronary application of either bone marrow–derived or blood-derived progenitor cells into the infarct-related artery (IRA) in 59 patients (Assmus et al., 2002). Similarly BOOST Trial also showed significant improvements in regional wall motion and global LVEF after BMMNCs infusion (Wollert et al., 2004).

In a study done by Nagaya et al. (2005), transplanted MSCs were engrafted into the myocardium in a rat model of DCM which improved cardiac function, possibly through induction of myogenesis and angiogenesis, as well as by inhibition of myocardial fibrosis (Nagaya et al., 2005). As so far no human clinical trials utilizing the potential of MSC in Dilated Cardiopathy patients have known to be reported, we can further expand our study by infusing autologous mesenchymal stem cells in future studies.

We should also explore the other ways of obtaining pluripotent cells for e.g. through stimulation of the bone marrow with granulocyte colony stimulating factor (G-CSF), and removal, through serial aphereses, of populations of stem cells present in the peripheral blood. (Fabio Vilas et al., 2004; Hyun et al., 2007; Li Zhan et al., 2007) and Umbilical Cord Blood which can be harvested after birth (Goldberg et al., 2007; Kai Hong et al., 2007; Kamihata et al., 2001).

Conclusion

Based on the results of the present study with regard to the improvement in the patient's clinical parameters, we would like to propose cell therapy as an alternative to be investigated for the treatment of severe dilated cardiomyopathy.

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