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Application of Stem Cell Technology for Coronary Artery Disease at the All India Institute of Medical Sciences, New Delhi, India

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ABSTRACT

Stem cell technology is rapidly gaining popularity as a way to improve the prognosis of patients with coronary artery disease and heart failure. In this review, we systematically analyze the basis, methods, and results of stem cell technology for coronary artery disease at the All India Institute of Medical Sciences, New Delhi, India.

INTRODUCTION

Myocardial infarction (MI) resulting from coronary artery disease leads to a loss of cardiomyocytes and the formation of scar tissue. This results in ventricular remodeling and is a cause of heart failure and death. Although the myocytes that are lost during MI cannot be regenerated, a small population of muscle cells in the region of viable myocardium may replicate and prevent heart failure [Quaini 2002]. At All India Institute of Medical Sciences (AIIMS), New Delhi, India, which is one of the apex, tertiary level referral centers of India catering to a large population of patients from all over India and nearby Asian countries, stem cell therapy is carried out for a wide range of cardiac and noncardiac conditions (Table 1). In this review, we briefly outline our experience with an ongoing project on the application of stem cell technology for coronary artery disease.

Basis for Stem Cell Transplantation

There is growing interest in stem cell transfer as a potential new methods of improving the prognosis of heart failure [Ertl 1993; Quaini 2002; Strauer 2002; Ramakrishnan 2003; Lunde 2006]. This approach is based on the assumption that left ventricular (LV) dysfunction is largely due to loss of a critical number of cardiomyocytes, which can be partly reversed by the implantation of new contractile cells into postinfarction scars [Ertl 1993; Quaini 2002; Strauer 2002; Ramakrishnan 2003; Lunde 2006]. It has been shown in animal experiments that transplantation of fetal cardiomyocytes or skeletal myoblasts may help to regenerate muscle cells and coronary vessels, but these are unable to perform the normal physiological functions. Implantation of bone marrow–derived multipotent stem cells has also been shown to induce this phenomenon and to improve cardiac function in experimental animal models [Orlic 2001a, 2001b; Tomita 2002].

Table 2 summarizes the various types of cells that can be used to induce myocardial regeneration. Cells that are capable of differentiating into cardiac myocytes include autologous skeletal myoblasts and bone marrow stem cells of autologous origin [Blau 2001; Goodell 2001; Krause 2001].

Totipotent cells have unlimited capacity. The totipotent cells of the very early embryo have the capacity to differentiate into extraembryonic membranes and tissues, the embryo, and all postembryonic tissues and organs. The pleuripotent stem cell is a single stem cell that has the capacity of developing cells of all germ layers (endoderm, ectoderm, and mesoderm). Pleuripotent cells can turn into any cell other than the placenta, whereas multipotent stem cells are stem cells that have the capability of developing cells of multiple germ layers . These cells sit atop a lineage hierarchy and can generate multiple types of differentiated cells, the latter being cells with distinct morphologies and gene expression patterns. The use of fetal cardiomyocytes raises ethical problems and acquisition concerns with the risk of rejection. Adult cardiomyocytes have the disadvantage of oncogenecity and acquisition concerns without rejection and ethical problems. Skeletal myoblasts of autologous origin have a high proliferative potential in vitro. However, when engrafted, they fail to express gap junctions and to retain organized contractile function because of a lack of electrical coupling [Perin 2004]. They pose no other concern. Embryonic stem cells carry ethical and acquisition problems with the risk of oncogenecity. One of the advantages from embryonic stem cells is the absence of HLA epitopes, so that rejection of the cells appears to be no real problem. Autologous marrow stromal cells are ideal as there are no such risks involved. The use of these cells has been reported by many recently. The markers for these stem cells are CD34+, Lin–, C-Kit+, and CD133+.

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Table 1. Stem Cell Therapy (n = 385)

Disease	Number of Patients
Myocardial infarction	43 (11.1%)
Dilated cardiomyopathy	60 (15.6%)
Muscular dystrophy	45 (11.7%)
Cerebral palsy	70 (18.1%)
Diabetes	15 (3.8%)
Nonunion fracture	33 (8.6%)
Peripheral vascular disease	9 (2.3%)
Extrahepatic biliary atresia	14 (3.6%)
Spina bifida	9 (2.3%)
Retina degeneration	16 (4.1%)
Spinal cord injury	15 (3.8%)
Amylotropic lateral sclerosis	33 (8.6%)
Motor neuron disease	21 (5.4%)
Ischemic stroke	2 (0.5%)

Table 2. Cellular Cardiomyoplasty: Cell Sources and Limitations

Cell Source	Ethical Problems	Acquisition Concerns	Rejection	Oncogenicity
Fetal cardiomyocytes	Yes	Yes	Yes	No
Adult cardiomyocytes	No	Yes	No	Yes
Skeletal myoblast	No	Yes	No	No
Embryonic stem cell	Yes	Yes	No	Yes
Marrow stromal cell	No	No	No	No

The cells can be delivered directly into the myocardium by direct injection at the time of coronary artery bypass grafting (CABG) or percutaneously by intracoronary injection or endocardial delivery or intravenous injection. Intravenous injection of these cells is not a very effective method as only a small fraction of the cells reach the myocardium because of seeding in other organs. These cells can be injected into the coronary arteries with the help of cardiac catheterization and selective cannulation of the desired coronary artery. This is a more efficient method of delivering these cells into the myocardium. However, these cells can be delivered more efficiently to the target areas by directly injecting them into the myocardium at the time of CABG. At our institute, we have routinely been injecting these cells by direct injection at the time of CABG via either a median sternotomy or via an anterolateral thoracotomy after harvesting the left internal mammary artery with the assistance of the da Vinci robotic system (Intuitive Surgical, Mountain View, CA, USA). It is also possible to inject these cells directly into the scarred areas of the myocardium during totally endoscopic CABG (TECAB). Our experience with TECAB is limited and we have not used this approach for stem cell injection. In 2 cases where we performed CABG using robotic assistance via a limited anterolateral thoracotomy, we successfully injected stem cells directly into the scarred myocardium. We believe that as more experience is gained with minimally invasive CABG and TECAB, these approaches will be used with increasing frequency for direct stem cell delivery into the infarcted myocardium.

Patient Selection, Technique of Isolation, Preparation, and Injection of Stem Cells

The project has been approved by the hospital ethics committee and detailed informed consent has been obtained from all patients. At our institute, we use autologous bone marrow cells for this purpose and we follow a strict protocol

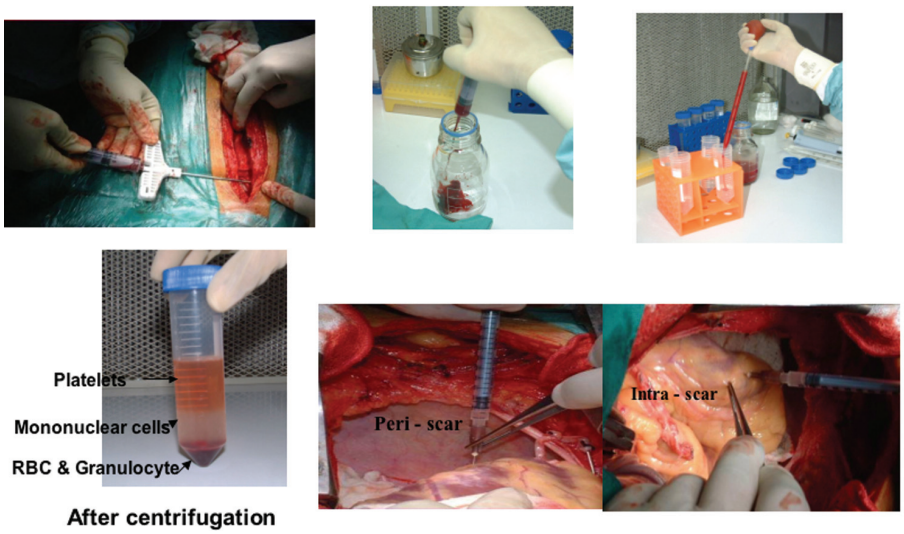

Figure 1. Bone marrow aspiration, collection, and injection.

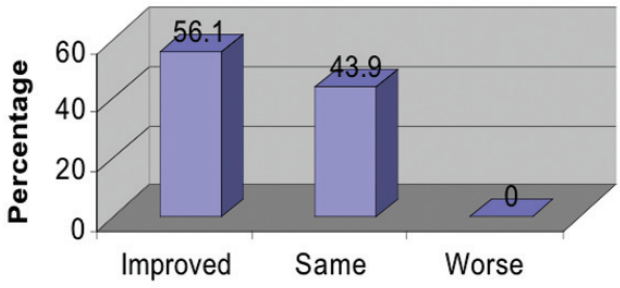

Figure 2. Effect on infarcted segments injected with stem cells: echocardiography analysis.

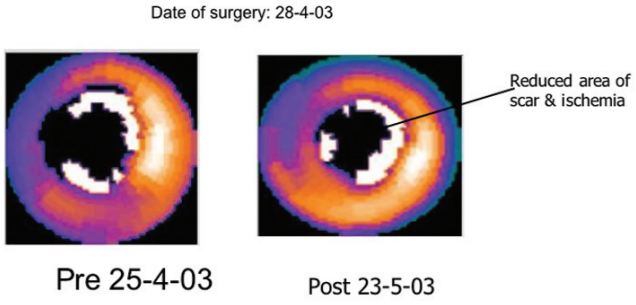

Figure 4. Stress thallium: before and after coronary artery bypass grafting + stem cell implantation.

for this procedure. Patients with coronary artery disease undergoing CABG and with reduced LV function with an area of nonviable myocardium are considered candidates for this procedure.

Preoperative work-up consists of 16-segment echocardiography, 20-segment stress thallium analysis, coronary angiography, and 5-segment analysis on left ventriculography. We have now added positron emission tomography (PET) scans for preoperative and postoperative evaluation of these patients.

The process of isolation of stem cells is fairly standard and has been described in detail previously [Strauer 2002]. After sternotomy, bone marrow is obtained by aspiration from both the sternal halves using a wide-bore syringe and needle. Typically, 30 to 40 mL of aspirate is obtained. This marrow is processed to isolate stem cells. Bone marrow is sent for stem cell preparation while CABG is performed. The bone marrow so obtained is diluted and centrifuged by the Ficoll density gradient centrifugation method to separate the stem cells. These are then washed and stained with trypan blue dye to test viability. Giesma staining is used to study the cells. Using a Neubauer chamber, a cell count is performed. These processed stem cells are injected in infarcted areas at the end of the CABG procedure, including nonrevascularizable areas (Figure 1).

Statistical Analysis

Data are reported as mean ± standard deviation and as percentages. Group comparison for continuous variables was performed by the Student *t* test and categorical variables by the Chi square test; $P < .05$ was deemed statistically significant.

RESULTS

At our institute, 43 patients underwent combined CABG and stem cell transplantation between February 2003 and October 2006. Their mean age was 51.6 ± 6.5 years (range, 42-62 years). Eighty-six percent had a documented previous MI, 43% had hypertension, 36% were chronic smokers, and 14% had diabetes. The basal New York Heart Association class ranged from 2 to 4 (mean, 2.9 ± 0.7). All patients had akinetic areas with 86% of the akinetic areas occurring in the anterior wall. The basal LV ejection fraction was 33% ± 16%. These patients received 2 to 4 grafts (mean, 2.8 ± 0.6). Additional procedures were Dor's procedure (n = 2), LV clot removal (n = 1), and post-MI ventricular septal defect closure (n = 1). As a matter of policy, we do not perform CABG in patients with acute MI unless they have persistent angina or hemodynamic compromise. If the patient was otherwise stable, CABG was usually performed at an interval of 6 weeks following the MI.

The mean volume of bone marrow aspirated was 34 ± 8 mL. The mean cell count was 15 ± 22 million per mL and

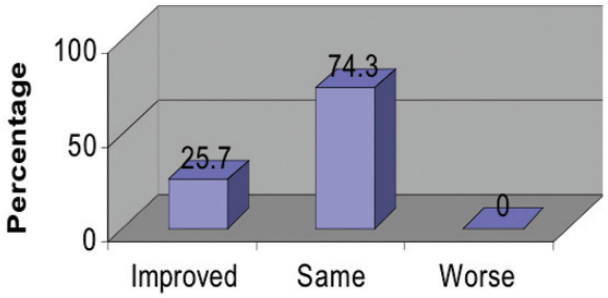

Figure 3. Effect on infarcted segments injected with stem cells: stress thallium analysis.

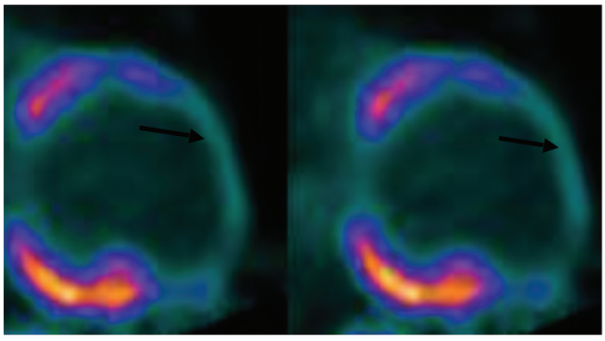

Figure 5. Positron emission tomography scan showing matched defect (scar).

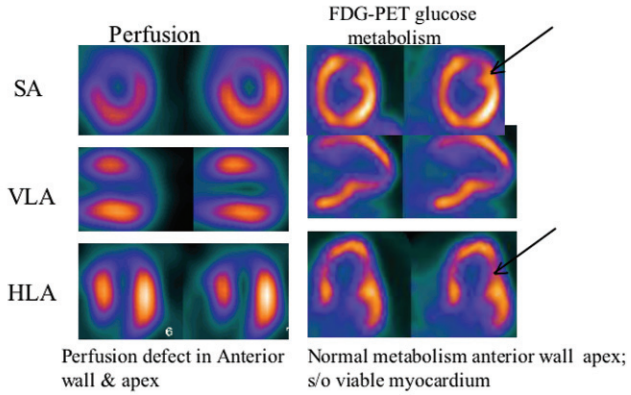

Figure 6. Positron emission tomography scan postprocedure showing viability.

the mean mononuclear cell percentage was 89% ± 2% with viability being 99% ± 1%. The mean final volume was around 12 ± 5 mL with a mean CD34 count of 1.21 ± 0.6. The number of injections per patient ranged from 25 to 48 and the amount injected was 0.25 cc/sq cm. The postoperative period was uneventful. There were no perioperative or postoperative deaths, and the hospital stay ranged from 6 to 11 days.

All patients underwent serial echocardiograms and stress thallium 20-segment analysis. In the last 5 patients, PET scans were obtained. The mean follow-up was 14.5 ± 3 months (range, 2-36 months). There was a significant improvement in New York Heart Association class from a baseline of 2.9 ± 0.7 to 1.25 ± 0.6 ($P < .001$). On echocardiography at the last follow-up, the LV end-diastolic dimensions remained stable at 143 ± 39 mL (versus 140 ± 37 mL at baseline; P = not significant). The LV end-systolic dimensions also remained stable at 86.5 ± 38 mL (versus 94 ± 32 mL at baseline; P = not significant). Echocardiographic LV ejection fraction analysis revealed evidence of improvement to 41% ± 9% (versus 32% ± 12% at baseline; $P = .05$). On 16-segment echocardiographic analysis, more segments showed improvement than deterioration overall. A mean 3.7 ± 2.6 segments showed improvement, 10.0 ± 1.6 segments showed no change, and 2.3 ± 2.6 segments showed worsening. Echocardiographic evaluation of injected infarcted segments (n = 4.1 ± 2.0) showed improvement in 2.3 ± 1.7, the others remained the same, and none worsened. Overall, 56.1% of infarcted areas injected with stem cells improved (Figure 2).

On stress thallium 20-segment analysis (Figures 3 and 4) at the last follow-up, the number of scarred segments reduced from 5.4 ± 2.7 to 4.6 ± 2.6, and the number of normal segments increased from 6.2 ± 3.9 to 8.2 ± 4.1. More segments showed improvement (5.8 segments improved, 14.2 showed no change, and none worsened).

On stress thallium evaluation of injected infarcted segments (n = 7 ± 2), 1.8 ± 1.9 segments showed improvement, the others remained same, and none worsened. Overall, 26% of injected infarcted segments improved. PET scans confirmed these findings (Figures 5 and 6). On Holter evaluation at follow-up, no significant arrhythmias were noted.

There is no direct evidence of myocardial revascularization following stem cell injection as these patients did not undergo follow-up coronary angiograms. However, the overall improvement in the ejection fraction along with a reduced area of the scar on stress thallium and PET analysis seem to indicate that neovascularization may at least partially be responsible for the observed benefits, particularly as there was no evidence of worsening of the already infracted areas. The time required to achieve these benefits can be at best speculative, but we have observed reduced areas of scarring as early as 1 to 2 months following surgery.

CONCLUSION

Bone marrow–derived stem cell transplantation during CABG is feasible and safe, and the bone marrow obtained from the sternum at the time of CABG provides an adequate number of stem cells. Our early data suggest that this procedure may be beneficial in patients with scars and/or a recent infarction in the LV. Further studies are needed to confirm this.

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