ASIAN
CARDIOVASCULAR & THORACIC
ANNALS

Intramyocardial Angiogenic Cell Precursors in Nonischemic Dilated Cardiomyopathy

Kitipan V Arom, Permyos Ruengsakulrach, Michael Belkin and Montip Tiensuwan

Asian Cardiovasc Thorac Ann 2009;17:382-388
DOI: 10.1177/0218492309338105

ORIGINAL ARTICLE

Intramyocardial Angiogenic Cell Precursors in Nonischemic Dilated Cardiomyopathy

Kitipan V Arom, PhD, Permyos Ruengsakulrach, PhD, Michael Belkin, MD[1], Montip Tiensuwan, PhD[2]

Cardiovascular Surgery, Bangkok Heart Hospital
Bangkok, Thailand
[1]Sackler Faculty of Medicine, Tel-Aviv University
Tel-Aviv, Israel
[2]Department of Mathematics, Faculty of Science, Mahidol University
Bangkok, Thailand

ABSTRACT

To determine the efficacy of intramyocardial injection of angiogenic cell precursors in nonischemic dilated cardiomyopathy, 35 patients with nonischemic dilated cardiomyopathy underwent injections of angiogenic cell precursors into the left ventricle (cell group). Seventeen patients with nonischemic dilated cardiomyopathy were matched from the heart failure database to form a control group that was treated medically. Angiogenic cell precursors were obtained from autologous blood, cultured in vitro, and injected into all free-wall areas of the left ventricle in the cell group. After these injections, New York Heart Association functional class improved significantly by 1.1 ± 0.7 classes at 284.7 ± 136.2 days, and left ventricular ejection fraction improved in 71.4% of patients (25/35); the mean increase in left ventricular ejection fraction was 4.4% ± 10.6% at 192.7 ± 135.1 days. Improved quality of life was demonstrated by better physical function, role-physical, general health, and vitality domains in a short-form health survey at the 3-month follow-up. In the control group, there were no significant improvements in left ventricular ejection fraction or New York Heart Association class which increased by 0.6 ± 0.8 classes. It was concluded that intramyocardial angiogenic cell precursor injection is probably effective in the treatment of nonischemic dilated cardiomyopathy.

Disclosures and Freedom of Investigation

Professor Michael Belkin is an advisory board member, a minor shareholder, and receives a consulting fee from TheraVitae Co. Ltd. However, the authors had full control of the study, methods used, outcome measurements, data analysis, and production of the written report.

(Asian Cardiovasc Thorac Ann 2009;17:382–8)

KEYWORDS: Adult Stem Cells, Cardiomyopathy, Dilated, Heart Failure, Hematopoietic Stem Cell Transplantation, Ventricular Dysfunction, Left

INTRODUCTION

Nonischemic dilated cardiomyopathy (DCM) is characterized by dilatation and impaired contractile function of the left ventricle (LV) or both ventricles. It may be idiopathic, familial/genetic, viral and/or immune, alcoholic/toxic, or associated with a recognized

---

Kitipan V Arom, PhD Tel: +662 3103323 Fax: +662 3103088 Email: karom@bangkokheart.com
Division of Cardiovascular Surgery, Bangkok Heart Hospital, 2 Soi Soonvijai 7, New Petchburi Road, Bangkok 10320, Thailand.
doi: 10.1177/0218492309338105

cardiovascular disease in which the degree of myocardial dysfunction is not explained by abnormal loading conditions or the extent of ischemic damage. Histology is nonspecific, and presentation is usually with heart failure that is often progressive. Arrhythmias, thromboembolism, and sudden death are common and may occur at any stage. Angiotensin-converting enzyme (ACE) inhibitors and beta blockers improve myocardial gene expression, symptoms, and long-term survival in nonischemic DCM, especially in the early stages. Implantation of a cardioverter-defibrillator significantly reduces the risk of sudden death from arrhythmia. Surgical options are limited, with heart transplantation being the definitive treatment, but the shortage of donor hearts limits its application. Cell transplantation has undergone extensive investigations and promises to improve health and quality of life by repairing or regenerating cells, tissues, or organs. Both embryonic and adult stem cells (somatic) are theoretically available, but the ethical issue with embryonic stem cells has limited their clinical use. Sources of adult cardiac stem cells include fetal cardiomyocytes, umbilical cord blood, bone marrow, peripheral blood, skeletal myoblasts, and resident cardiac progenitor cells. Bone marrow is not only a site of hematopoiesis but also serves as an important reservoir of mature granulocytes and stem cells, including hematopoietic stem cells, mesenchymal stem cells, and fibrocytes. Hematopoietic stem cells have the potential to generate cardiomyocytes and vascular cells, and these have been identified in both adult heart and peripheral tissues. In-vivo experiments have suggested that these progenitor cells, including endothelial progenitor cells, are capable of replacing damaged myocardium and vascular tissues.[1] They have been used widely with safety and a good early outcome in the treatment of myocardial infarction and ischemic cardiomyopathy.[2] Skeletal myoblasts, however, have been shown to increase the risk of arrhythmia. There is currently little information on stem cell implantation in nonischemic DCM.[3,4] The objective of this study was to assess the efficacy and short-term results of direct intramyocardial injection of peripheral blood stem cells in patients with nonischemic DCM.

PATIENTS AND METHODS

Between May 2005 and July 2007, 35 patients with nonischemic DCM (cell group) underwent intramyocardial autologous stem cell injection. These patients were treated at our institution from the start of this project, in conjunction with the University of Pittsburgh. Prior to commencing cell therapy, they had all undergone medical treatment and surgical/device procedures, which had failed; patients who might benefit from any conventional treatments were excluded. The study was approved by the ethics committee and institutional review board. Informed consent was obtained before the procedure. The patients were screened for severe contagious infections to exclude HIV and hepatitis. Other exclusion criteria were malignancy within the previous 3 years and current therapy with anticancer, cytotoxic, or immunosuppressive medication. All patients had a recent coronary angiogram (within 6 months) confirming the absence of coronary artery disease, and the preoperative workup included routine chest radiography, electrocardiography, and N-terminal pro brain natriuretic peptide levels. Echocardiography without stressing and/or cardiac magnetic resonance imaging (MRI) were performed in all cases. Cardiac MRI was conducted (except in patients with contraindications such as metallic implants) with a 3.0-Tesla scanner (Achieva 3.0T system with Philips Quasar Dual gradients; Philips Medical Systems, The Netherlands). All patients completed a quality of life assessment using a multipurpose short-form health survey (SF-36), a 6-min walk test, New York Heart Association (NYHA) functional class evaluation, and routine laboratory tests required for general anesthesia. Within the same time frame, 17 matched patients from our heart failure database, who were treated by medical means only, served as controls (Table 1).

The adult stem cells used in this study were angiogenic cell precursors developed by VesCell technology (TheraVitae Co. Ltd., Ness Ziona, Israel).[5] The angiogenic cell precursors were obtained from the patient's own blood, avoiding immunological concerns. A 250-cc peripheral blood sample was collected using the technique for general blood donation, and sent for

Table 1. **Characteristics of patients with nonischemic dilated cardiomyopathy**

Variable	Cell Group ($n = 35$)	Controls ($n = 17$)	p Value
Age (years)	56.7 ± 14.3	57.5 ± 14.3	0.8
NYHA class	3.0 ± 0.6	2.7 ± 1.0	0.2
Preoperative LVEF	23.9% ± 6.5%	25.0% ± 8.9%	0.6
Pulmonary hypertension	11 (31.4%)	1 (5.9%)	0.08
Diabetes mellitus	11 (31.4%)	6 (35.3%)	1
Hypertension	11 (31.4%)	3 (17.6%)	0.3
ACE inhibitor	10 (28.6%)	1 (5.9%)	0.08
Digitalis	11 (31.4%)	5 (29.4%)	1

ACE = angiotensin-converting enzyme, LVEF = left ventricular ejection fraction, NYHA = New York Heart Association.

cell expansion. Blood cultures for aerobic and anaerobic bacteria were collected at the same time, and negative results were confirmed during the process. The multipotent progenitor cells were isolated from the blood, rich in CD45, $\mathrm{CD31^{Bright}}$, $\mathrm{CD34^{+}CD45^{-/Dim}}$, and $\mathrm{CD34^{Bright}}$ cells, at a concentration of $1.5\text{–}3.0 \times 10^{6}$ $\mathrm{cells \cdot mL^{-1}}$, and cultured under sterile conditions with vascular endothelial growth factor (R&D Systems, Minneapolis, MN, USA) and $5\,\mathrm{U \cdot mL^{-1}}$ heparin (Kamada, Beit-Kama, Israel). The process of cell expansion took 5 days. The number and viability of the cells were checked and passed the quality control test before use. The final product consisted of over 1.5 ± 0.5 million autologous endothelial progenitor cells suspended in 15 mL of sterile cell culture medium. Acceptable culture parameters, assessed by microscopy and flow cytometry, were cell viability $\geq 75\%$ and morphology showing spindle-shaped large cells forming long thread-like structures. Sterility tests were performed according to the Code of Federal Regulations Title 21 §610.12 on a sample of the cell fraction supernatant or phosphate buffered saline (PBS) following cell washing. Interim negative sterility results of all samples taken at different stages of culture were compulsory before release of the final product. Bacterial endotoxin tests were undertaken according to the US Pharmacopeia 23. The Limulus amebocyte lysate test was carried out on a sample of supernatant taken from the cell culture. Endotoxin levels below the acceptable limits were compulsory prior to release of the final product. Gram staining was used as a rapid and qualitative method to assess bacterial contamination of tissue culture samples. A negative result of Gram staining of samples taken from the cell washing medium before vialing was compulsory before release of the final product. Mycoplasma contamination was also excluded. The product phenotype was assayed by immunostaining, angiogenic potential (tube formation assay), and cytokine secretion. All cell preparations complied with predefined release criteria for safety and potency.

Cell samples were washed in PBS, re-suspended in 100 μL of PBS, stained with specific fluorochrome-conjugated antihuman antibodies or isotype-matched nonspecific controls, and incubated in the dark for 30 min on ice. Cell suspension triplicates of 500,000 cells each were stained, assessed by a fluorescence-activated cell sorter (FACSCalibur, Becton Dickinson), and analyzed by CellQuest Pro software (Becton Dickinson). The percentage of each marker was determined, and the mean and percentage coefficient of variance was calculated for each test tube. The results were expressed as mean ± standard error of the percentage of stained cells. The number of stained cells was calculated by multiplying the number of harvested cells by the staining percentage obtained using fluorescence-activated cell sorting. The total number and percentage of angiogenic cell precursors as well as CD34+ hematopoietic stem cell markers 31–33 were determined using flow cytometry. Harvested cells were tested for CD31, a molecule expressed by progenitor cells as well as mature angiogenic and endothelial cells, and uptake of acetylated low-density lipoprotein, a molecule rapidly taken up and internalized by endothelial and angiogenic cells that is regularly used for their characterization. Angiogenic cell precursors were defined as cells exhibiting high levels of CD31 ($\mathrm{CD31^{Bright}}$) concomitant with uptake of acetylated low-density lipoprotein. Briefly, harvested cells were incubated on ice in the dark for 30 min with specific fluorochrome-conjugated CD34-angiogenic cells (BD Biosciences, San Jose, CA, USA), CD31-PE (BD Biosciences) or with isotype-matched nonspecific controls. The angiogenesis potential of the cells was measured by their ability to form 3-dimensional tube-like structures according to a widely used scale, using an in-vitro angiogenesis assay kit (Chemicon) and scoring under an inverted light microscope (Nikon ECLIPSE TS-100). Cytokine secretion was assessed in samples of the harvested cells washed in PBS, re-suspended to one million in 1 mL of X-Vivo 15, and grown for 24 h in 24-well plates. Cytokine secretion in the supernatant was compared to that of the medium only, using flow cytometry and a human angiogenesis kit (Becton Dickinson). The number of cells prior to injection was 33.7 ± 35.9 million (range, 3.3–200 million). The cells were injected into all areas of the LV in the cell group.

Under general anesthesia with one-lung ventilation, the patient was placed in the right lateral decubitus position. A 1-cm incision was made in the left chest at the 5th intercostal space on the posterior axillary line. A thoracoscope was placed through this incision, and the chest cavity was examined. Two other instrument access ports were introduced at the 3rd and 7th intercostal spaces on the posterior axillary line. The thoracoscope and appropriate instruments were used to open the pericardium longitudinally, anterior and posterior to the phrenic nerve. This technique was accomplished with ease when the left pleural and pericardial cavities had never been invaded before. Care must be taken to free the pericardium from the heart without injury to the phrenic nerve, which could be detrimental in such seriously ill patients. The thoracoscopic instruments and pericardial traction stitches were placed appropriately to assist in reaching all regions of the LV wall. The cell injection was performed using a 23-guage butterfly needle with a homemade guard. The needle was placed in the heart and the injection was achieved manually outside the chest with an extension line. There were 30 sites of injection of 0.5 mL. The cells were injected into the all areas of the LV except the interventricular septum. After adequate hemostasis, the openings were closed, and a small chest drain was left at the 7th intercostal space.

Both the control and cell groups received medical treatment according to the standard heart failure guidelines of the Joint Commission International disease-specific program and the ACC/AHA 2005 Guideline Update for Diagnosis and Management of Chronic Heart Failure in the Adult, by their own cardiologists. Medications that effect the natural history of the disease, especially neurohormonal antagonists (ACE inhibitors, beta blockers, aldosterone antagonists) were part of the treatment strategy. Each medication was started at the appropriate NYHA class. Adverse drug reactions were monitored and managed accordingly. Medications with potentially deleterious effects in heart failure (most calcium channel blockers, antiarrhythmics class 1A and 1C, nonsteroidal antiinflammatory drugs, and Cox 2 inhibitors) were avoided. All patients were followed up in the same manner, with a repeat of the preoperative measurements at 1, 3, 6, and 12 months in the cell group.

Statistical analyses were carried out with SPSS for Windows version 10.0 software (SPSS, Inc., Chicago, IL, USA). Continuous preoperative clinical data of the two groups were compared with Student's t test, and categorical variables with chi-square and Fisher's exact tests. The chi-square test was used to calculate probability values for comparison of dichotomous variables. Fisher's exact test (2-sided) was used when the number in any cell was less than 5. Continuous variables are expressed as the mean ± standard deviation, unless otherwise indicated. Categorical data are reported as proportions. Correlations between pairs of continuous variables were evaluated using Spearman's correlation coefficient. The paired t-test was used to compare differences in LV ejection fraction (LVEF) and NYHA class between the preoperative and postoperative periods. A p value of less than 0.05 was considered significant.

RESULTS

There were no major adverse cardiac events such as neurological deficit, excessive postoperative bleeding, new renal insufficiency, or new pulmonary failure. There were no malignant arrhythmias (ventricular fibrillation, recurrent sustained ventricular tachycardia, torsades de pointes ventricular tachycardia in long QT syndrome, or ventricular tachycardia associated with unstable hemodynamics) in the perioperative period. Six patients died during the 2 years of follow-up; the 2-year survival was approximately 72%. All 6 patients had a sudden death due unknown causes. At 284.7 ± 136.2 days after the injection, NYHA functional class improved by 1.1 ± 0.7 ($p < 0.001$). In contrast, NYHA functional class did not improve significantly in the control group (2.45 ± 0.9 pre-medical treatment vs. 1.9 ± 0.5 post-medical treatment, $p = 0.052$). All patients in the cell group tolerated cardiac rehabilitation very well. The 6-min walk tests improved at the 3-month follow-up (369.5 ± 122.4 m postoperatively vs. 425 ± 218.5 m postoperatively, $p = 0.2$). N-terminal pro brain natriuretic peptide levels decreased by 1,471.7 ± 2,648.5 $\text{pg} \cdot \text{mL}^{-1}$ at 5–7 days after surgery (3,907.0 ± 3,735.23 $\text{pg} \cdot \text{mL}^{-1}$ preoperatively vs. 2,435.32 ± 2,526.3 $\text{pg} \cdot \text{mL}^{-1}$ postoperatively, $p = 0.1$) and by 2,100.4 ± 3,382 $\text{pg} \cdot \text{mL}^{-1}$ at 3 months (3466.3 ± 3654 $\text{pg} \cdot \text{mL}^{-1}$ preoperatively vs. 1365.9 ± 1041.1 $\text{pg} \cdot \text{mL}^{-1}$ postoperatively, $p = 0.2$). The overall decrease in N-terminal pro brain natriuretic peptide was 1,946.2 ± 3394.1 $\text{pg} \cdot \text{mL}^{-1}$ at 84.4 ± 190.7 days ($p = 0.02$). LVEF was higher in 71.4% of patients (25/35) who underwent cell injection. The overall LVEF improved by 4.4% ± 10.6% ($p = 0.02$) from 23.9% ± 6.5% to 28.3% ± 10.7% at 192.7 ± 135.1 days (Figure 1). There was a moderate positive correlation between the number of cells and the improvement in LVEF ($p = 0.02$, Spearman's correlation coefficient = 0.47). LVEF did not improve in the medically treated group: it was 27.6% ± 7.6% after medical treatment compared to 25.0% ± 8.9% before treatment ($p = 0.2$). The quality of life assessed by SF-36 at the 3-month follow-up (Figure 2) demonstrated significant improvements in physical function ($p = 0.004$), role-physical ($p = 0.02$), general health ($p < 0.001$), and vitality domains ($p = 0.007$).

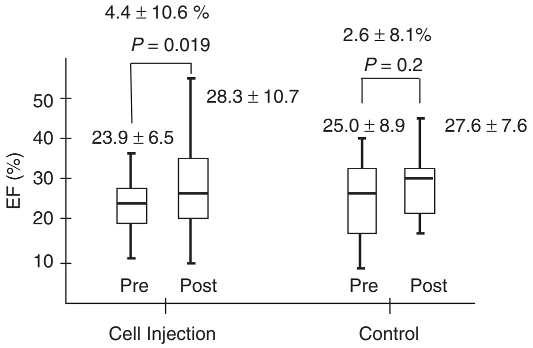

Figure 1. Box plot of left ventricular ejection fraction (EF) before and after injection of angiogenic cell precursors (left pair) and before and after medical treatment (right pair). The upper and lower limits of boxes indicate the first and third quartiles of the data (50% lies within the box). The median is shown by a line inside the box. The ends of the vertical lines indicate the minimum and maximum values, unless outliers are present, in which case they extend to a maximum of 1.5 times the inter-quartile range.

DISCUSSION

Although this was a nonrandomized case-matched study, the patients were not selected and comprised all those

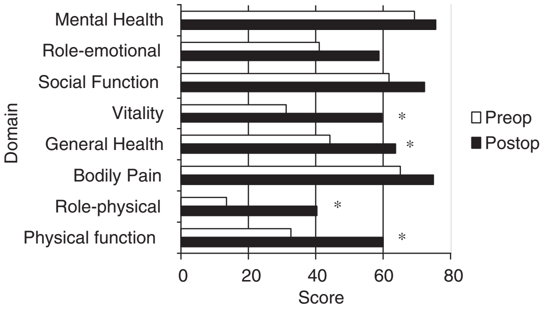

Figure 2. The quality of life scores before and after injection of angiogenic cell precursors assessed at the 3-month follow-up.

referred to us for stem cell therapy for nonischemic DCM. Cell transplantation has been investigated in both animal models and the clinical setting of nonischemic DCM. Transplanted ventricular myocytes or autologous smooth muscle cells improved contractile function in myopathic hearts in animals.[3] Clinical studies of cell implantation in nonischemic DCM are limited. Huang and colleagues[6] performed percutaneous coronary autologous bone marrow mononuclear cell transplantation in 10 patients and found it improved the 6-min walk test, decreased the re-hospitalization rate, and increased exercise capacity; however, LVEF and LV size were unchanged compared to control and pretreatment values. Wang and colleagues[7] reported similar results after intracoronary injection of autologous mesenchymal stem cells in 12 patients with nonischemic DCM. Seth and colleagues[4] noted improved NYHA class and LVEF (by 5.4%) after intracoronary autologous bone marrow mononuclear cell transplantation in 24 patients with nonischemic DCM.

So far, bone-marrow stem cells have been used most often for clinical investigations. Bone marrow is composed of multiple cell types such as hematopoietic, mesenchymal, and stromal stem cells, as well as other cell types yet to be characterized. Endothelial progenitor cells exist in bone marrow as well as in the systemic circulation. These cells have 3 surface markers (CD34, CD133, and vascular endothelial growth factor-2) and properties of embryonal angioblasts which contribute to re-endothelization and neovascularization. Highly enriched Lin-c-kit+ adult bone marrow cells regenerated functional myocardium within an infarcted region in mice.[8] The bone marrow mesenchymal cell marker CD29 showed contraction and ultrastructural features of sarcomere formation after co-culturing with neonatal rat ventricular myocytes.[9] The proposed mechanisms of heart function improvement are protection of at-risk myocytes against apoptosis, and induction of myocyte proliferation/regeneration.

Adult peripheral blood CD34+ cells can transdifferentiate into cardiomyocytes, mature endothelial cells, and smooth muscle cell in animal models in vivo.[1,10] Both cell fusion and transdifferentiation may account for the transformation of peripheral blood CD34+ cells into cardiomyocytes in vivo.[11] However, Gruh and colleagues[12] found no convincing evidence of transdifferentiation of endothelial progenitor cells into cardiomyocytes when co-culturing neonatal rat cardiomyocytes. Koyanagi and colleagues[13] demonstrated an important role of E-cadherin in endothelial progenitor cell differentiation into cardiomyocytes. The endothelial progenitor cells can transiently form ultra-fine intercellular connections with neonatal rat cardiomyocytes. This transient cell fusion resulting in the exchange of macromolecular complexes may contribute to the cell fate change of endothelial progenitor cells during co-culture. The angiogenic cell precursors used in this study were generated from autologous peripheral blood and represent a heterogenic stem/progenitor cell population containing both CD34 and CD117 multipotent hematopoietic cells that can potentially differentiate in vivo in response to tissue signals at the site of injection, and lineage-specific angiogenic precursors. It has been shown that human angiogenic cell precursors, delivered via intramyocardial or intracoronary injection in an ischemic cardiomyopathy rat model, were engrafted into damaged cardiac tissue and improved cardiac function within 4 weeks through effects on scar morphology and blood vessel formation.[14] Fractional shortening, functional area contraction, and LVEF improved significantly compared to controls. LV end-diastolic volume was reduced, and higher maximum +dP/dt and lower minimum –dP/dt values were demonstrated in the cell injection groups by pressure-volume measurements. The myocardial scar area on computed planimetry at 4 weeks after cell transplantation was significantly reduced in the intramyocardial cell injection group compared to controls, but not in the intracoronary injection group. Antibodies against human mitochondria protein were used to quantify implanted cell survival and to identify the distribution and engraftment of implanted human angiogenic cell precursors. Cells expressing human mitochondria were observed in the ischemic myocardial area at 4 weeks after implantation. The density of surviving angiogenic cell precursors was greater in the ischemic zone after intramyocardial injection than after intracoronary injection. Antibodies against myosin heavy chain and troponin I revealed some muscle-like cells in the ischemic myocardial area, while non-myogenic cells were observed in the ischemic area of controls. Antibodies against factor VIII indicated higher blood vessel density in the myocardial ischemic area than in controls.

Roura and colleagues[15] demonstrated defective vascularization and impaired vasculogenesis (de-novo vascular organization of mobilized endothelial progenitors) and angiogenesis in patients with dilated cardiomyopathy. The defective vascularization was associated with reduced myocardial expression of vascular β-catenin, an important angiogenic regulator. Werner and colleagues[16] also showed that endothelial progenitor cell transfer was effective in attenuating myocardial damage in a model of dilated cardiomyopathy. The angiogenesis effect of mechanical puncture of the myocardium was demonstrated by Chu and colleagues;[17] however, this was controversial. These studies formed the rational for the use of intramyocardial angiogenic injection of cell precursors in our study. This also has the advantage of better cell retention; significantly more cells were retained in the heart after intramyocardial injection (11.3% ± 3%) compared to the trans-coronary approach (2.6% ± 0.3%).[18] We have previously shown the feasibility and safety of transplanting cells by this minimally invasive approach.[19] We noted that both endoscopic and micro-invasive approaches allowed access to all areas of the LV wall with minimal difficulty, and the injection could be carried out with ease and negligible complications.

Although LVEF improved in 71% of patients, the mean LVEF improved by only 4.4%, with a large standard deviation (10.6%) indicating substantial variations in response. We are not yet be able to predict which patients will benefit from the cell injection. The improvement of 4.4% was comparable with previous reports on bone marrow stem cell injection for nonischemic DCM. Even when LVEF did not improve, most patients were in a better NYHA functional class with enhanced quality of life.

Magnetic resonance imaging is a useful tool for the evaluation of LV function, but it was carried out in only 10 (28.6%) patients preoperatively as most of them had defibrillator or cardiac resynchronization therapy. Only 3 patients had follow-up MRI at our institution. Of the 6 patients who died during follow-up, 5 had improved LVEF (by 2%–7%); in the other, LVEF fell by −0.6%. Unfortunately, they all died suddenly overseas and the details were unavailable. Concerning medical treatment, ACE inhibition contributes to an improvement in LV geometry and myocyte contractile function by increased extracellular support from the collagen matrix and normalization of the beta-adrenergic receptor system. ACE inhibitors have shown clinical and survival benefits in heart failure and also in nonischemic DCM. They were used less in the control group (5.9%) than in the cell injection group (28.6%), but there was no significant difference.

This was a non-randomized study of matched cases and not a level I study by any means, but it is the best we can do at the present time; the severity of illness in the patients who want to be treated does not allow a double-blind study at this stage. The efficacy observed suggests that the use of optimal medical treatment combined with cellular therapy might be beneficial in end-stage nonischemic DCM before heart transplantation. A further development would be to identify the type of cell with the highest efficacy and ease of harvesting and expanding. The next generation of induced pluripotent stem cells may provide more potent cells for alleviating heart failure.[20] The dose-response needs to be evaluated, and the scientific basis is required to explain the results. Further limitations were that LVEF was followed up by either MRI or echocardiogram or both; therefore, there may be some small discrepancies in the evaluations. As 27 (77%) patients who had intramyocardial angiogenic cell precursor injections were from the USA, the tests were not completed at every follow-up point. Nevertheless, we obtained at least one follow-up for each of the 35 patients (100%). This report used only the latest follow-up results which may have reflected the best possible outcome of the treatment. However, the NYHA functional class and quality of life data were completed directly by the patients.

It was concluded that transthoracic intramyocardial autologous angiogenic cell precursor transplantation is feasible and safe. Early results in the cell-treated group were better than in the medically treated controls, with improvements in quality of life, functional class, and ejection fraction. Long-term results are necessary, and randomized double-blind placebo-controlled trials are needed to confirm the efficacy of this treatment. We hope that this report will benefit members of the stem cell research community who are interested in the treatment of nonischemic DCM, and stimulate further investigations.

ACKNOWLEDGMENT

The authors thank Vibul Jotisakulratana, MD, Vitoon Pitiguagool, MD, Sujit Banyatpiyaphod, MD, Piyapan Pamornsing, MD, Chockchai Suwanakijboriharn, MD, Sawat Asavapiyanond, MD, and Chayanin Vatcharasiritham, MD for their assistance with the surgery.

Presented at the *88th Annual Meeting, American Association for Thoracic Surgery*, San Diego, California, USA, May 10–14, 2008.

REFERENCES

1. Badorff C, Brandes RP, Popp R, Rupp S, Urbich C, Aicher A, et al. Transdifferentiation of blood-derived human adult

endothelial progenitor cells into functionally active cardiomyocytes. Circulation 2003;107:1024–32.

2. Lunde K, Solheim S, Aakhus S, Arnesen H, Moum T, Abdelnoor M, et al. Exercise capacity and quality of life after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: results from the Autologous Stem cell Transplantation in Acute Myocardial Infarction (ASTAMI) randomized controlled trial. Am Heart J 2007;154:710.e1–8.
3. Ohno N, Fedak PW, Weisel RD, Mickle DA, Fujii T, Li RK. Transplantation of cryopreserved muscle cells in dilated cardiomyopathy: effects on left ventricular geometry and function. J Thorac Cardiovasc Surg 2003;126:1537–48.
4. Seth S, Narang R, Bhargava B, Ray R, Mohanty S, Gulati G, et al. Percutaneous intracoronary cellular cardiomyoplasty for nonischemic cardiomyopathy: clinical and histopathological results: the first-in-man ABCD (Autologous Bone Marrow Cells in Dilated Cardiomyopathy) trial. J Am Coll Cardiol 2006; 48:2350–1.
5. Porat Y, Porozov S, Belkin D, Shimoni D, Fisher Y, Belleli A, et al. Isolation of an adult blood-derived progenitor cell population capable of differentiation into angiogenic, myocardial and neural lineages. Br J Haematol 2006;135:703–14.
6. Huang RC, Yao K, Li YL, Zhang YQ, Xu SK, Shi HY, et al. Transplantation of autologous bone marrow mononuclear cells on patients with idiopathic dilated cardiomyopathy: early results on effect and security. Zhonghua Xin Xue Guan Bing Za Zhi 2006;34:111–3.
7. Wang JA, Xie XJ, He H, Sun Y, Jiang J, Luo RH, et al. A prospective, randomized, controlled trial of autologous mesenchymal stem cells transplantation for dilated cardiomyopathy. Zhonghua Xin Xue Guan Bing Za Zhi 2006;34:107–10.
8. Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Transplanted adult bone marrow cells repair myocardial infarcts in mice. Ann N Y Acad Sci 2001;938:221–30.
9. Li X, Yu X, Lin Q, Deng C, Shan Z, Yang M, et al. Bone marrow mesenchymal stem cells differentiate into functional cardiac phenotypes by cardiac microenvironment. J Mol Cell Cardiol 2007;42:295–303.
10. Yeh ET, Zhang S, Wu HD, Korbling M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. Circulation 2003;108:2070–3.
11. Zhang S, Wang D, Estrov Z, Raj S, Willerson JT, Yeh ET. Both cell fusion and transdifferentiation account for the transformation of human peripheral blood CD34-positive cells into cardiomyocytes in vivo. Circulation 2004;110:3803–7.
12. Gruh I, Beilner J, Blomer U, Schmiedl A, Schmidt-Richter I, Kruse ML, et al. No evidence of transdifferentiation of human endothelial progenitor cells into cardiomyocytes after coculture with neonatal rat cardiomyocytes. Circulation 2006;113:1326–34.
13. Koyanagi M, Urbich C, Chavakis E, Hoffmann J, Rupp S, Badorff C, et al. Differentiation of circulating endothelial progenitor cells to a cardiomyogenic phenotype depends on E-cadherin. FEBS Lett 2005;579:6060–6.
14. Sun Z, Wu J, Fujii H, Wu J, Li SH, Porozov S, et al. Human angiogenic cell precursors restore function in the infarcted rat heart: a comparison of cell delivery routes. Eur J Heart Fail 2008;10:525–33.
15. Roura S, Planas F, Prat-Vidal C, Leta R, Soler-Botija C, Carreras F, et al. Idiopathic dilated cardiomyopathy exhibits defective vascularization and vessel formation. Eur J Heart Fail 2007;9:995–1002.
16. Werner L, Deutsch V, Barshack I, Miller H, Keren G, George J. Transfer of endothelial progenitor cells improves myocardial performance in rats with dilated cardiomyopathy induced following experimental myocarditis. J Mol Cell Cardiol 2005;39:691–7.
17. Chu VF, Giaid A, Kuang JQ, McGinn AN, Li CM, Pelletier MP, et al. Thoracic Surgery Directors Association Award. Angiogenesis in transmyocardial revascularization: comparison of laser versus mechanical punctures.. Ann Thorac Surg 1999;68:301–8.
18. Hou D, Youssef EA, Brinton TJ, Zhang P, Rogers P, Price ET, et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation 2005;112(Suppl I):150–6.
19. Arom KV, Ruengsakulrach P, Jotisakulratana V. Intramyocardial angiogenic cell precursor injection for cardiomyopathy. Asian Cardiovasc Thorac Ann 2008;16:143–8.
20. Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. Cell Stem Cell 2007;1:39–49.

Intramyocardial Angiogenic Cell Precursors in Nonischemic Dilated Cardiomyopathy

Kitipan V Arom, Permyos Ruengsakulrach, Michael Belkin and Montip Tiensuwan

Asian Cardiovasc Thorac Ann 2009;17:382-388
DOI: 10.1177/0218492309338105