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Direct Myocardial Implantation of Human Fetal Stem Cells in Heart Failure Patients

Y.V. Baltaytis

Institute for Regenerative Medicine, Villa Nova, St. John, Barbados

Heart failure (HF) is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood. It results from coronary artery disease in about two-thirds of patients; the remainder have non-ischemic cardiomyopathy, the cause of which may be known (e.g., hypertension, valvular disease, or myocarditis) or unknown (e.g., idiopathic dilated cardiomyopathy). A relatively common disorder, HF is estimated to affect nearly 5,000,000 people in the U.S., with about 550,000 new patients each year. Morbidity and mortality rates are high: each year in the U.S., HF results in approximately 970,000 hospitalizations and 53,000 deaths, and is a likely contributing factor in 265,000 deaths. The one-year mortality rate of patients in Class III to IV of the New York Hearth Association (NYHA) is nearly 40%. The cost of medical treatment for HF has been projected to be 27.9 billion dollars in the U.S. in 2005.

Cardiac transplantation is currently the only established surgical treatment for refractory end-stage HF (stage D), but it is available to fewer than 2500 patients in the United States each year. Other standard treatments of HF are limited to measures that only slow its progression or manage its symptoms, and include various pharmacological therapies and surgical interventions.

In preclinical studies, cell therapy has been shown to regenerate myocardial cells in the injured or necrotic myocardium, stimulate angiogenesis, and improve both systolic and diastolic ventricular function. In patients with myocardial infarction, autologous stem cell therapy has been shown to stimulate angiogenesis, repair local cardiac tissue, and improve cardiac function. In patients with ischemic HF, this therapy has been demonstrated to improve ejection fraction, heart pumping action, quality of life, NYHA class, and exercise capacity, and has recently been shown to improve ejection fraction in patients with non-ischemic cardiomyopathy as well. In both ischemic and non-ischemic patients, bone-marrow-derived stem cell therapy has been shown to be safe in terms of arrhythmias and/or other adverse events.

Stem cells can be derived from three main sources: adult tissues, e.g., bone marrow; blastocysts (embryonic stem cells); and tissues of fetuses from terminated ectopic pregnancies, elective abortions or spontaneous miscarriages. Most cell therapy administered to HF patients to date has been bone-marrow-derived adult autologous stem cells. Human fetal-derived stem cells (HFDSCs) are thought to be more pluripotent than adult stem cells, i.e., the former can develop into a wider range of specialized cells. Although HFDSCs have been used to treat a variety of conditions (blood and immune system disorders, spinal cord injuries, stroke, other neurological and eye disorders, and diabetes, there have been no reports of HFDSCs used in HF therapy.

Given the promising findings to date of autologous stem cell therapy in HF patients, the possibly greater differentiating potential of HFDSCs and their successful application in treating a variety of other disorders, this trial was designed to investigate the safety and efficacy of HFDSC implantation for the treatment of idiopathic cardiomyopathy.

Materials and methods

This was an open-label, single-arm, prospective, clinical study performed at Luis Vernaza Hospital, Guayaquil, Ecuador. The study was approved by the Ethics Committee of the hospital, and informed consents fully explaining the potential risks of the surgical procedure and HFDSC transplantation were obtained from the patients.

Patient Population. All patients were assessed at baseline for biochemistry profile, CBC, coagulation profile, electrocardiogram, chest X-ray, transthoracic echocardiogram with General Electric Vivid 7 device, cardiac catheterization with coronary angiogram to exclude ischemic heart disease, 6-minute walk test over a 30 meters flat surface, exercise tolerance test under Naughton protocol modified, NYHA classification and Minnesota CHF test.

Patients participating in the study met the following inclusion criteria:

–AHA diagnostic criteria for dilated non-ischemic, non-chagasic cardiomyopathy

–Ejection fraction < 35% by transthoracic echocardiography

–NYHA functional class III or IV

–Bilirubin, creatinine, BUN, serum glucose, GOT, GPT<2.5 times normal values

–Symptomatic despite optimal drug therapy for HF

Exclusion criteria included:

–Valvular heart disease requiring surgical treatment

Адрес для корреспонденции: Baltaytis Y.V., Institute for Regenerative Medicine, Villa Nova, St. John, Barbados; e-mail: ulbalt@gmail.com

– Other concurrent life-threatening disease, infectious disease, blood disease, diagnosis of epilepsy, or positive on HIV or VDRL testing

– Intolerance or hypersensitivity to biological substances

– Participation in another clinical trial

– History of drug or alcohol abuse, psychiatric disturbances or suicide attempts in the past 2 years

– Renal failure needing dialysis

– White blood cell count <5,000 or >12,000, hematocrit < 30%, pulmonary thromboembolism within 6 months

– Mechanical ventilation support in last 10 days

– Morbid obesity

Patients who were initially included who were noncompliant with the protocol (tests or treatments), which were lost to follow-up, or who developed an unrelated new illness were excluded from the study.

For each patient, preoperative medications (digoxin, furosemide, spironolactone, Ace-Inhibitors or Angiotensin Receptor Blockers and Beta- Blockers) were maintained throughout the study and follow-up.

*Stem Cells.*HFDSCs were provided by the Institute for Regenerative Medicine (IRM, Villa Nova, St. John, Barbados), and processed and prepared by the Institute for Problems of Cryobiology and Cryomedicine (IPCC) in the Ukraine. IPCC obtains HFDSCs from fetuses 5-12 weeks gestation from legally consented, non-compensated donors who have undergone terminated ectopic pregnancies, elective abortions or spontaneous miscarriages. The HFDSCs are prepared from harvested fetal liver tissue under sterile conditions, and undergo polymerase chain reaction (PCR) testing for HIV, hepatitis B and C, mycoplasma, toxoplasmosis, cytomegalovirus, herpes simplex I and II, rubella, and Treponema pallidum; and culture tests for bacterial and fungal contamination. The cell preparations are stored in cryoperservatives at –196°C in liquid nitrogen. The percentage of viable cells according to the IPCC certification was 60%.

HFDSCs were shipped in a cyropreserved state at –150 to – 196°C in Minishipper containers from IPCC to Luis Vernaza Hospital for this study, and maintained in this state until use. Just prior to the procedure, the HFDSCs cells were thawed to room temperature and in 9 patients diluted in 80 ml of saline solution at 37є C; in one patient approached by minithoracotomy, the dilution was in 15 ml. Each patient received 60-80 million HFDSCs according to the information issued by the provider (The Institute for Regenerative Medicine).

Anesthesia and Surgical Technique. Patients were anesthetized with fentanyl 0.50 mcg/kg as a premedication, Thiopental 2mg/kg as induction, atracurium 1mg/kg for relaxation, and remifentanil 0.025 mcg/kg/min and sevofurane at 0.5-1.5% for maintenance during the procedure. Nine patients had midline sternotomy, and one had a left anterior minithoracotomy in the fifth intercostal space. Prior to the injections, 80 marks, 1 cm apart, were made with a blue methylene marker on the anterolateral, posterolateral, and diaphragmatic left ventricular wall, and anterolateral right ventricular wall, avoiding coronary blood vessels.

A total of 80 1 ml injections 3 mm deep were administered by a 25-gauge needle with a catheter in the marked areas. For the patient undergoing the minithoracotomy (SB, female, 48 years), only 15 injections were made in the anterolateral wall. During the procedure, patients were monitored for arterial pressure, central venous pressure, urine output, EKG, O_2 saturation, and $ETCO_2$. Potassium (20 mEq/hour) and Magnesium (1g/hour) infusions were started before the operations and maintained up to the chest closure. All patients were extubated on the theater.

At 30, 90 and 210 days after the procedure, each patient was reassessed for NYHA classification, ETT; ejection fraction, LVEDD and 6-minutes walk test performance. The Minnesota CHF test was performed before the operation and at 210 days.

Statistical Analysis. Mean values for parameters just before and after the procedure were compared using paired t-tests (Primer program).

Results and discussion

Six female and 4 male patients (age range 47-77 years) met the inclusion criteria and participated in the study. Five had functional impairment of NYHA class IV and 5 were of class III.

There was no operative or perioperative mortality. One male patient (UJ, 69 years), during the procedure but before receiving injections, had a transient single intraoperative ventricular fibrillation, which was terminated by electrical cardioversion. One male (MJ, 66 years) and one female (VM, 77 years) required temporary pacemakers postoperatively due to severe bradycardia (<40 bpm), for 24 hours and 48 hours, respectively. The former patient received dobutamine for 24 hours. He also had a mild pericardial effusion at 3 weeks, which resolved spontaneously. He was later excluded from the trial for non-compliance (abandoned his controls) and he finally died at 5 months. The heart autopsy showed nests of cardiomyocites among the fibrotic tissue but it was not possible to determine whether they were new myocardium growing or the remaining native fibers. The immunochemistry search of the heart showed the expression of the cell markers CD 34, CD117, Actine smooth muscle, Vimentin and Myogenin, and increased neovascularization in adjacent fibrotic tissue. The electron microscopy did not clarify if the new fibers were self-regenerating heart cells or

due to the implants. Two Polymerase Chain Reactions searches for chimerism were negative.

One female patient (QA, 52 years) had a right hemiparesis 3 days after implantation due to ischemic stroke, confirmed by CT scan. Although she was alive and recovering as of this writing, she was excluded from the protocol because she was unable to perform the follow-up tests of the study.

Another female patient (BM), was hospitalized 3 times (at 2, 4 and 7 months) because of abdominal pain due to severe gastroenteritis that led to transient decompensation; she therefore required inotropic support with dobutamine for 48, 48 and 72 hours respectively. While there was no improvement in her EF at 210 days, there was a significant improvement in exercise tolerance test, LVEDD and 6 minute walk test. At 210 days follow up, no other complications were observed.

The 8 patients who provided 30-90 and 210 days follow-up data demonstrated improvements clinically and on imaging studies. With regard to imaging studies, increased wall thickness both eccentric and concentric was noted in association with an increased contractility in those regions. Compared to baseline assessments, patients improved in NYHA class (mean + SD: 3.4 + 0.5 to 1.9 + 0.6); LVEDD by transthoracic echocardiography decrease of 6mm (6.71 + 0.54 cm to 6.11 +0.58 cm, 8.94%, p= 0.001) ; Minnesota CHF score decrease from 74 + 24 to 6+7; EF as assessed by transthoracic echocardiography (26.6 + 4.0% to 34.8 + 7.2% a 31 % increase, (p=0.001); ETT from 4.25 min to 16.63 (291.3% increase, p= 0.0001) and 2.46 to 5.63 METS (128.9 % increase, p= 0.0001), (Table 6); 6-minute walk test : 251.0+113.1 seconds to 360.0+ 0 seconds, a 43.42 % increase, (p = 0.003); average distance: 284.4+144.9 m to 468.2+ 89.8 m, a 64.4 % increase, (p=0.007).

Current treatment options for refractory end-stage HF are limited in effectiveness, and no current treatment can totally repair ischemic or necrotic myocardial tissue. Studies have suggested that autologous stem cell therapy can be beneficial in improving cardiac function in patients with HF due to coronary diseases and, recently, in patients with non-ischemic idiopathic cardiomyophathy. The preliminary findings from this study constitute the first report of the application of HFDSC therapy in HF patients. We found statistically significant improvement in left ventricular function (EF and LVEDD), NYHA class, Exercise Tolerance Test, 6-minutes walk test performance and Minnesota test 210 days following direct myocardial cell implantation, including in the patient who received the injection in the anterolateral wall via minithoracotomy. All these patients were maintained on their preoperative medications and doses throughout the study.

It is worth noting that no new recurrent or permanent arrhythmias were seen after implantation in this surgical series of patients. As reviewed by others, arrhythmias have been reported in studies of patients with HF or myocardial infarction given skeletal myoblast cell therapy, but this complication appears to be less of a problem with autologous adult stem cells.

The improvements seen in our patients could have been due to the administered HFDSCs or the surgical procedure itself. Past studies with autologous adult stem cells have shown ejection fraction improvements in control groups given only plasma, however, the improvements were small (5%). In the non-control group in contrast, ejection fraction increased by as much as 35%. The improvement seen in the present cohort suggest that the HFDSCs have some therapeutic effect. The mechanism of action as seen via echocardiographic imaging suggests, however preliminarily, that the increased wall thickness and contractility in the regions described might be due to an increase in the number of cardiomyocytes. More studies are needed to validate this hypothesis and to evaluate for the presence of a DNA from the donor.

Whether or not these cell markers expression and the electron microscopy are showing if those phenomena are constant or just appeared in a patient who did not get as a good outcome as the rest of the cohort remain as a question that also requires further investigation to be answered.

We recognize that the relatively small number of patients may represent a significant limitation of this study. These initial findings, however, suggest that HFDSC transplantation improves cardiac function in HF patients early on and is sustained at 7 months. No rejection reactions or malignancy were seen as of this writing. We believe that given the sustained effect of HFDSC therapy it indicates that offers another possibility in treating advanced HF patients, and represents a new approach that could be used before other major surgical treatments, including heart transplantation, due to their availability of having them on the shelf avoiding the time consuming autologous bone marrow harvesting and process. Irrespective of the improvement seen in this trial, it is still premature to determine accurately its mechanism of action, indications and doses, so more research is needed

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