Surgical Myocardial Implantation of Human Fetal Stem Cells in Heart Failure Patients.

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Antecedentes
Aunque el uso de células madre fetales humanas se estudiaron en diversas enfermedades, ningún estudio clínico se ha presentado para el tratamiento de de la insuficiencia cardiaca con ellas.
Diseñamos este protocolo para determinar la factibilidad y seguridad del uso de células madre provenientes de preparaciones de hígado fetal en pacientes con Miocardiopatia Dilatada.

Métodos y Resultados
Diez pacientes con insuficiencia cardiaca debido a Miocardiopatia Dilatada fueron implantados quirúrgicamente, sin mortalidad perioperatoria, con preparaciones de células madre fetales conteniendo: CD34+ =0.89 +/- 0.09%; CD45+ = 2.33 +/- 0.25%; CD34 + CD45+ 0.82+/- 0.09%; Gly-A + = 90.0+/- 2.54 y CDU33+ = 0.39 +/- 0.07%, 70% de las cuales co-expresando CD34.
Ningún cambio se hizo en medicación pre-operatoria. No se manifestaron enfermedades malignas ni reacciones de rechazo.
A 7 meses de su implante los pacientes mostraron la factibilidad y seguridad de las células madre utilizadas y mejorías respecto de sus controles pre-operatorios en clase funcional NYHA (3.5 +/- 0.5 a 2+/-0.7, p = 0.0001); disminución del volumen de fin de diástole del VI de 5.2 ml. (6.67 +/- 0.64 a 6.09 +/- 0.55, 7.8%, p = 0.001); Score de test de Minnessota disminuyendo de 74.9 +/- 22.4 a 14.6 +/- 27.2, p = 0.0001; incremento de FE 27.0 +/- 3.9% a 35.0 +/- 6.2%, 30% de incremento, p = 0.005, acompañado por crecimiento excéntrico o concéntrico de espesor de paredes miocárdicas en regiones cuya contractilidad aumentó; mejora en el Test de Tolerancia al Ejercicio (ETT) 2.35 +/- 0.9 a 5.63 +/- 1.3 METS (139.6%, p = 0.0001); aumento de valores en el Test de Marcha de 6 Minutos desde 237.0 +/- 113 segundos a 360.0 +/- 0, 51.9%, p = 0.01 y distancia de 275 +/- 152 metros a 467.5 +/- 90 metros, 70%, p = 0.004.

Conclusión
La implantación de células madre fetales humanas, es factible, segura y mejora la función cardiaca en pacientes con Miocardiopatia Dilatada a 7 meses. Mayores estudios clínicos, número de pacientes y tiempo de seguimiento son necesarios para confirmar estas observaciones.

Palabras clave: *Angiogénesis, Miocardiopatía Terapia Celular, Falla Cardiaca, Miogénesis.*

Subject heads
CV surgery; transplantation; ventricular assistance; cardiomyopathy; congestive heart failure; angiogenesis; myogenesis; stem cells.

Word count
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Abstract
Background – Although human fetal-derived stem cells (HFDSCs) have been studied in the treatment of a variety of conditions, no clinical studies have been reported to date on their use in treating HF. We sought to determine the feasibility and safety of HFDSC treatment in patients with HF, injecting human fetal liver stem cells preparations.
Methods and Results – 10 patients with HF due to dilated cardiomyopathy were surgically implanted with HFDSC (CD34+ cells = 0.89 ± 0.09%; CD45+ cells = 2.33 ± 0.25%.; CD34+CD45+ cell fraction 0.82 ± 0.09%; Gly-A+ cells 90.0 ± 2.54% and CD133+ 0.39 ± 0.07%, 70% of the latter co-expressing CD34) without preoperative mortality.

No changes were done in their preoperative medications. No malignancies or rejections reactions were found. Patients who provided 7 months follow up demonstrated the safety and feasibility of HFDSC and improvements at 210 days vs. baseline in NYHA class (mean+SD: 3.5+0.5 to 2+0.7, p= 0.0001; a diminution in end diastolic dimension of 5.2 mm (6.67+0.64 cm to 6.09 + 0.55 cm, 7.8%, p= 0.001; Minnesota CHF score decrease from 74.9 + 22.4 to 14.6+27.2, p=0.0001; ejection fraction (27.0+3.9% to 35.0+6.2%) a 30 % increase, p=0.005, that was accompanied by eccentric or concentric thickening of the myocardium in regions with increased contractility; ETT from 4.25+1.8 min to 16.63+4.2 (291.3% increase, p= 0.0001) and 2.35+0.9 to 5.63+1.3 METS (139.6 % increase, p= 0.0001), 6-minutes walk test : time from 237.0+113 seconds to 360.0+0 seconds, a 51.9 % increase, (p = 0.01); average distance: 275+152 m to 467.5 + 90 m, a 70 % increase, p=0.004

Conclusion –Implantation of HFDSCs is feasible, safe and improves cardiac function in HF patients at 210 days, more clinical research is required to confirm these observations.
Key words: angiogenesis, cardiomyopathy, cell therapy, heart failure, fetal stem cells, myogenesis.

Introduction

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).1 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.2 The one-year mortality rate of patients in Class III to IV of the New York Hearth Association (NYHA) is nearly 40%.3 The cost of medical treatment for HF has been projected to be 27.9 billion dollars in the U.S. in 2005.2 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.1

In preclinical studies, cell therapy has been shown to regenerate myocardial cells in the injured or necrotic myocardium and induced cardiomyopathy as well, stimulate angiogenesis, improve both systolic and diastolic ventricular function.4-16 Cardiomyocyte-like cells differentiated from human embryonic stem cells were transplanted into the left ventricular wall of pigs with complete heart block. The transplanted cells reestablished the cardiac rhythm17-18
In patients with myocardial infarction, autologous stem cell therapy has been shown to stimulate angiogenesis, repair local cardiac tissue, and improve cardiac function. 19-24 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.25-27
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. 27
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, 28-33 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 feasibility and safety of HFDSC implantation for the treatment of idiopathic cardiomyopathy.

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 way of implanting, 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 coronary 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 cardiomyopathy1
• Ejection fraction < 35% by transthoracic echocardiography
• NYHA functional class III or IV
• Bilirrubin, creatinine, BUN, serum glucose, GOT, GPT < 2.5 times normal values
• Symptomatic despite optimal drug therapy for HF
Exclusion criteria included:
• Valvular or coronary heart disease requiring surgical treatment
• 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 and dosages (digoxin, furosemide, spironolactone, Ace-Inhibitors or Angiotensin Receptor Blockers and Beta- Blockers) were maintained throughout the study and follow-up.

Cells isolation and cryopreservation

HFDSCs were provided, processed and prepared by the Institute for Problems of Cryobiology and Cryomedicine (IPCC) in the Ukraine. IPCC obtains HFDSCs from liver tissues from fetuses 5-12 weeks gestation from legally consented, non-compensated donors who have undergone terminated ectopic pregnancies, elective abortions or spontaneous miscarriages. The tissue was obtained under full Ethical Committee approval at the public Institute for Problems of Cryobiology and Cryomedicine in Kharkov, Ukraine. Fetal liver was disrupted using a fine gauge sterile stainless steel mesh, followed by repeated aspiration via syringe through a range of needles (19G – 25G). The single cell suspension thus formed was centrifuged at 1000g for 10min and the cell pellet resuspended in freezing medium. A serum-free sucrose based medium was used in the study containing 5% DMSO as a penetrating cryoprotectant. The cells were cooled down to -40_C with a low (-1_C/min) rate, with equilibration at -27_C, then down to –80_C with high (-10_C/min) rate, after which they were plunged into liquid nitrogen. The thawing was performed on 37_C water bath. The viability of the cell preparations was determined staining with trypan blue using haemocytometer.39 47 The percentage of viable cells was 65+7 % and the average amount was 70 +5,000,000 cells according to the result of 70 consecutive samples checked before and also assessed to this trial.

Characteristics of the cells

The type of cells used in the present study has been previously described.40 Briefly, the specific cell phenotype was determined using CD34, CD133, CD45 and glycophorin-A (Gly-A markers. The proportion of cell types analyzed in a human fetal liver preparation was: CD34+ cells = 0.89 ± 0.09%, CD45+ cells = 2.33 ± 0.25%. The CD34+CD45+ cell fraction comprised 0.82 ± 0.09%, Gly-A+ cells comprised 90.0 ± 2.54% and CD133+ cell content was 0.39 ± 0.07%. The majority (>70%) of the CD133+ cells co-expressed CD34.

The HFDSCs are prepared 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. 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.

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 sevoflurane at 0.5-1.5% for maintenance during the procedure. Just prior to the procedure, the HFDSCs cells were thawed to room temperature and in 9 patients approached through sternotomy they were diluted in 80 ml of saline solution at 37_ C; in one patient who did not accepted sternotomy being approached by minithoracotomy in fifth left intercostal space, the dilution was in 15 ml.
Before 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 in the 9 patients who underwent sternotomy.
A total of 80 1 ml injections 3 mm deep were administered intramyocardially by a 25-gauge needle with a catheter in the marked areas. For the patient approached by minithoracotomy (SB, female, 48 years), only 15 marks and injections were made in the anterolateral wall. During the procedure, patients were monitored for arterial pressure, central venous pressure, urine output, EKG, O2 saturation, and ETCO2. 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.

Follow up

At 30, 90 and 210 days after the procedure, each patient was reassessed for NYHA classification, ETT; EF, 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 210 days after the procedure were compared using paired t-tests (Primer program).

Results

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 (Table 1).
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 moderate pericardial effusion at 3 weeks, which resolved spontaneously. He abandoned his controls and medication and was rehospitalized at 90 days with a decompensated atrial fibrillation. His echocardiogram showed an EF of 28 % (baseline 35%) and a moderate to severe pericardial effusion .He improved with amiodarone and was discharged 48 hs later. He again abandoned his medication and control and he finally died at 5 months for severe hypoproteinemia and HF.
His heart autopsy findings were: 30% occlusion of left descending and right coronary arteries, chronic

subendocardial transmural infarction in anterior wall, myocardial hypertrophy and dilation, chronic pericarditis and focal aortic and mitral calcifications. Microscopically: severe interstitial fibrosis, angiogenesis, areas with large capillary population in the middle of fibrosis, and many areas with big nuclei and mitosis. The electron microscopy revealed an unusual spherical shape of a large population of mitochondrions with trilaminar crests aligned along the nuclei of the myocites, sarcoplasm reticulum less abundant than usual, terminal specializations at Z lines, and few T tubules were found. 2 consecutive Polimerase Chan Reaction tests were negative for chimerism. Markers CD 117, CD 34, Vimentin, Actine smooth muscle and Myogenin expressed positively.

One female diabetic type II patient (QA, 52 years) had a right hemiparesis 3 days after implantation due to ischemic stroke, confirmed by CT scan. She used to suffer from frequent hypoglycemic episodes after taking her oral hypoglycemic agents with no control, as she did it that time. After being walking along her room she lied in bed and the next morning she was found in hypoglycemic comma (blood glucose 42mg %), her arterial pressure being 60/40 mmHg. Although she was alive and recovering as of this writing, she is unable to perform the whole follow-up tests according to the protocol.

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. At 210 days it was demonstrated improvements clinically and on imaging studies (Tables 1-7). 4 patients showed slight increase in IgE blood levels at 7 months suggesting unspecific allergies. C3, C4, IgA, IgG, IgM, Anti-DNA, Anti-nuclear Antibody, C Reactive Protein and white blood cells count were normal in all patients except transient leukocytosis in the female patient (BM) as she underwent her abdominal infections. No liver or renal dysfunctions or skin rushes were seen. None of the tumoral markers searched: CEA, Alfa-fetoprotein, CA 125, CA 19-9, CA 72-4, CA 15-3, CYFRA 21-1, PSA total and free, expressed positively in any of them.

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 (Table 1), patients improved in NYHA class (mean + SD): 3.4 + 0.5 to 2 + 0.7, p= 0.0001 (Table 2); LVEDD by transthoracic echocardiography decrease of 5.2 mm (6.67 + 0.64 cm to 6.09 + 0.55 cm, 7.8%, p= 0.001 (Table 3); Minnesota CHF score decrease from 74.9 + 22.4 to 14.6+27.2, p=0.0001 (Table4); EF as assessed by transthoracic echocardiography (27.0 + 3.9% to 35.0 + 6.2% a 30 % increase, p=0.005, (Table 5); ETT from 4.25+ 1.8 min to 16.63 + 4.2 (291.3% increase, p= 0.0001) and 2.35 + 0.9 to 5.63 + 1.3 METS (139.6 % increase, p= 0.0001) (Table 6); 6-minute walk test : time from 237.0+113 seconds to 360.0+ 0 seconds, a 51.9 % increase, (p = 0.01); average distance: 275 +152 m to 467.5 + 90 m, a 70 % increase, p=0.004 (Table 7).

Discussion

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 diseases19-23 and, according to our own experience (unpublished), 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.

As we aimed to prove feasibility and safety of those cells implants, and not having antecedents of cardiac injections in humans we started performing sternotomies to show the whole heart and get the possibility of injecting directly intramyocardium to reach as much walls as possible. We also considered it as a safer approach in such environment in case any accident happened. We performed left mini thoracotomy in one patient who did not accept sternotomy. 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,27 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.
Myocardial biopsy could not been performed since the patients did not give their consent.

The improvements seen in our patients could have been due to the administered HFDSCs or the surgical procedure itself but what was seen in the present cohort suggests 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 also to evaluate for the presence of a DNA from the donor. 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 is feasible, safe and improves cardiac function in HF patients early on and is

sustained at 7 months. No rejection reactions or malignancy were clinically seen as of this writing. We believe that given the sustained effect of HFDSC therapy it indicates that may 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. Regardless of the improvement seen in this trial and the fact of being the first world wide surgical implantation of fetal stem cells, it is still premature to come to a definitive conclusion, so further clinical research is needed

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