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Surgical Implantation of Stem Cells in Heart Failure Patients due to Idiophatic Cardiomyopathy

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Abstract

Between 2004 and 2005, thirteen patients with idiopatical cardiomyopathy were surgical implanted with two different types of Stem Cells. Ten patients were implanted with Human Fetal-Derived Stem Cells (HFDSCs) and three patients with Autologous Bone Marrow Stem Cells (ABMSCs).

Nine patients underwent a midline sternotomy, prior to the injections, 80 marks (1cm apart) were made with a blue methylene marker on the anterolateral, posterolateral, and diaphragmatic left ventricular walls and on the anterolateral right ventricular wall, avoiding coronary blood vessels. We administered 80 injections of 1mL each in the marked areas. The injections were 3mm deep with a 25-gauge needle and a catheter.

Three patients received Autologous Bone Marrow Stem Cells (ABMSCs) and one Human Fetal-Derived Stem Cells (HFDSCs) with minimally invasive approach.

We applied aliquots of 1cc separated by 2 to 3cm and 3 to 5mm in depth with a 25G needle with an average of 25 injections in the ABMSCs patients. The patient who received HFDSCs was injected only 15 times in the anterolateral wall.

The patients with HFDSCs improved in association with increased contractility in these regions. Compared with baseline assessments, we noted other improvements: The mean (±SD) NYHA class decreased from III/IV, ±0.5 to I/III±0.5 (P=.001); the mean EF increased 31%, from 26.6%±4.0% to 34.8% ± 7.2% (P=.005); performance in the ETT increased 291.3%, from 4.25 minutes to 16.63 minutes (128.9% in metabolic equivalents, 2.45 to 5.63) (P<.0001); the mean LVEDD decreased 15%, from 6.85±0.6cm to 5.80±0.58cm (P<.001); mean performance in the 6-minute walk test increased 43.2%, from 251±113.1 seconds to 360±0 seconds (P=.01); the mean distance increased 64.4%, from 284.4±144.9m to 468.2±89.8m (P =.004); and the mean result in the Minnesota congestive HF test decreased from 71±27.3 to 6±5.9 (P<.001) The Kaplan-Maier probability test of survival at 40 months' time was 66%. Rejection was not observed. These patients have not developed malignant nodules or cancer at all in the follow-up.

Patients with ABMSCs have a preoperative NYHA functional class of III/IV. Six months after receiving the stem cell treatment the average functional class value was I/III, showing a marked clinical improvement (p<0.05). There was a similar change in ventricular diameters: After 6 months the LVESV went from 50 mm to 42mm (p<0.05). After 24 months, two of the patients still maintained this improvement, while the third patient returned to the earlier values after suffering from pneumonia.

After 150 months two patients were alive and both received a Resynchronization Therapy. The third patient died of sudden death at 124 months' time.

Regardless of the improvement seen in this study, it is still premature to determine accurately the mechanism of action, indications, doses and type of stem cells needed. Therefore, it is imperative and extremely important that more research is done.

Keywords: Stem cells in heart failure patients; Idiophatic myocadiophaty treat with stem cells; Autologous stem cells; Embriofetal stem cells

Introduction

Congestive heart failure is one of the main causes for cardiologic morbility and mortality in the XXIst century [1,2]. Patients in advanced stages (NYHA functional classes III/IV) have an average of a 5-year survival rate below 50%, with an annual mortality of 40–50% [3], with high rates of re-hospitalization, morbidity and complications, and high related costs for health services. Etiology for dilated cardiomyopathy is 60% due to ischemic cardiomyopathy and 40% to idiopathic – non ischemic origin.

Patients have been treated with ACE inhibitors, diuretics, beta-blockers, spirolactone, ventricular re-synchronization, ventricular assistance and heart transplantation. For many years, heart transplantation has been the surgical treatment of choice for patients with advanced heart failure. This procedure has been successful in many countries; however it presents many limitations, the most important ones being the scarcity of donors and the contraindications of advanced age and severe co-morbid situations [4]. Moreover, there have been frequent deaths during the prolonged periods in the waiting list for organ reception.

The final stage of several heart diseases ends in congestive heart failure in the quantitative deficiency of cardiomyocytes and cardiac remodeling [5]. Reversion of cardiac remodeling lies in the possibility of myocyte regeneration and neo-vascularization of affected areas. The goal of cellular therapy is the re-population of the myocardium with cells capable of restoring contractility and blood flow: This will improve the systo-diastolic function of the heart. The injected cells must have the capacity to differentiate themselves into cardiomyocytes or promote revascularization.

Several studies have shown that the adult bone marrow is a rich reservoir of these pluri-potential, mesenchymal stem cells, which contribute to functional neo-angiogenesis. They also participate in wound healing and reversion of lower limb ischemia [6], post – MI neo-angiogenesis [7,8], endothelization of vascular grafts [9],

atherosclerosis [10], retinal and lymphoid neo-vascularization [11] and vascularization during neo-natal development [12].

Most cell therapy administered to HF patients to date has been bone-marrow-derived stem cells (BMDSCs) which are thought to be more pluripotent than adult stem cells: the former can develop into a wider range of specialized cells (13). Although HFDSCs have been used to treat various conditions, including blood and immune system disorders (14), spinal cord injuries (15), stroke (16), other neurological and eye disorders (17), and diabetes (18), there have been no reports of the use of HFDSCs in HF therapy.

Counting on the promising effects of ABMDSCs and HFDSCs we designed a trial to investigate the safety and efficacy of the implantation of these stem cells for the treatment of idiopathic cardiomyopathy.

This is the first pilot clinical study to assess the safety and feasibility of HFDSC in humans.

Thirteen patients were implanted: three patients were implanted with ABMDSC by minimally invasive surgical technique in March 2004 in Montevideo, Uruguay, and ten patients were implanted with HFDSCs by using two different surgical techniques: minimally invasive technique (one patient) and full sternotomy technique (nine patients) between January and February of 2005 in Guayaquil Ecuador.

The HFDSCs were obtained from fetuses of 5 to 12 weeks' gestation from legally consented, free donors who have undergone terminated ectopic pregnancies, elective abortions, or spontaneous miscarriages.

At that gestation period, totipotent stem cells' fetus haven´t developed the HLA histocompatibility complex yet, so there is no possibility of antigenicity between donor and recipient.

Methods

This study was performed under the authorization of the Hospital authorities and ethics council; and the patients' informed and signed consent.

The patients presented dilated idiopathic cardiomyopathy with a severe decrease in LVEF and functional capacity.

Inclusion criteria

- Patients in NYHA functional class III/IV.
- Dilated, idiopathic, non-ischemic and non-chagasic cardiomyopathy with LVEF <35%
- Optimal medical treatment including ACE inhibitors, spironolactone, beta-blockers and diuretics at an average 85% of the maximum dose
- Bilirrubin, creatinine, blood urea nitrogen, serum glucose, glutamine-oxaloacetic transaminase (aspartate aminotransferase), and glutamic-pyruvic transaminase level <2.5 times normal values
- Symptomatic condition despite optimal drug therapy for HF

Exclusion criteria

- Congestive heart failure decompensated in the last 6 days.
- Cancer present during the last 5 years.
- Presence of hematological diseases
- Leukocyte count above 12000/cc or below 5000/cc
- Renal failure requiring hemodialysis
- Previous cardiac surgery
- Valvular heart disease requiring surgery
- Preoperative steroid therapy
- Infectious disease
- Blood disease
- Diagnosis of epilepsy
- Positivity in Human Immunodeficiency Virus or venereal Disease Research Laboratory testing
- Intolerance or hypersensitivity to biological substances
- Participation in another clinical trial
- Having a record of drug or alcohol abuse
- Psychiatric disturbances
- Suicide attempts in the previous 2 years.
- Renal failure needing dialysis
- Pulmonary thromboembolism within the previous 6 months
- Mechanical ventilation support within the previous 10 days
- Morbid obesity

The patients included in this study were thirteen: ten treated with HFDSCs and three with ABMDSCs. Only one patient was excluded from the group of HFDSCs because he was non-compliant with the protocol test.

For each patient, preoperative medications (digoxin, furosemide, spironolactone, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers) were maintained throughout the study and the follow-up.

Stem cell extraction and processing

Bone marrow stem cells: Patients were taken to the Operating Room (OP) monitored, anesthetized and placed in the prone position. Bone marrow (BM) was harvested by the team hematologists from both sides of the iliac crests, as is habitually done in the hematology and hemotherapy departments. Using this technique we were able to harvest 500 to 600mL of bone marrow with a minimum number of punctured sites and were placed in a special container with 10000 U of heparin and acetyl salicilate lysine to prevent coagulation. At least 250mL of bone marrow must be harvested to continue with the protocol. The bone marrow was filtered with a 200-um filter. The resulting solution was centrifuged at 400g for 15 min. The cellular pellet was resuspended in phosphate-buffered saline solution (PBS). The cell solution was mixed 3:1 with a solution of 155 mmol/L NH_4Cl and 0.1 mmol/L EDTA and set for 15 min at room temperature. The solution was then centrifuged at 400g for 10 min. The pellet was washed with PBS and resuspended. The cell suspension was placed over a Ficoll-Paque (1.077 density) 4:1 and centrifuged at 400g for 30 min. The upper layer was aspirated, leaving the mononuclear cell layer at the interphase. The interphase cells were transferred to a new conical tube with PBS and centrifuged at 300g for 10 min. The supernatant was completely removed, and the cell pellet was resuspended in PBS. Cell counts were performed, and magnetic labeling with Isolex 300i was carried out according to standard protocol for peripheral blood progenitor cell products to obtain an enriched product of, at least, 70% CD_3^{4+} cells. The resulting cell solution was resuspended in 30 cc of each patient's own serum and 10 000 U of heparin sulfate. Cell viability was established with a standard Trypan blue exclusion.

Human fetal-derived stem cells: HFDSCs were processed and prepared by the Institute for Problems of Cryobiology and

Cryomedicine (IPCC) (Kharkov, Ukraine). The IPCC obtained HFDSCs from fetuses of 5 to 12 weeks' gestation from legally consenting free donors who have undergone terminated ectopic pregnancies, elective abortions, or spontaneous miscarriages. The HFDSCs were prepared from harvested fetal liver tissue under sterile conditions and undergo polymerase chain reaction testing for human immunodeficiency virus, hepatitis B and C, mycoplasma, toxoplasmosis, cytomegalovirus, herpes simplex viruses I and II, rubella, and Treponema pallidum. HFDSCs were also submitted to culture tests for bacterial and fungal contamination. Cell preparations are stored at –196°C in liquid nitrogen. The percentage of viable cells before thawing was 60% according to the IPCC certification.

The IPCC shipped HFDSCs in minishipping containers in a cryopreserved state (–150°C to –196°C) to Luis Vernaza Hospital in Guayaquil Ecuador for this study, and they were maintained in this state until use. Just before the procedure, HFDSCs were thawed at room temperature. In 9 patients, the cells were diluted in 80mL of saline solution at 37°C. In 1 patient, who underwent the procedure via a minithoracotomy approach, the cells were diluted in 15mL. Patients received $7.5x10^5$cells /mL and $5.3x10^6$cells /mL respectively.

Surgical technique

Sternotomy: Nine patients underwent a midline sternotomy and were injected HFDSCs (Figure 1). Prior to the injections, 80 marks (1cm apart of each other) were made with a blue methylene marker on the anterolateral, posterolateral, and diaphragmatic left ventricular walls and on the anterolateral right ventricular wall, avoiding coronary blood vessels. We administered 80 injections of 1mL each in the marked areas. The injections were 3mm deep with a 25-gauge needle and a catheter. During the procedure, patients were monitored for arterial pressure, central venous pressure, urine output, electrocardiogram, oxygen saturation, and end-tidal carbon dioxide concentration in the expired air. Infusions of potassium (20mEq/hour) and magnesium (1g/hour) were started before the operations and were maintained until the time of chest closure. All patients were extubated in the operating room.

Minimally invasive: Three patients received Autologous Bone Marrow Stem Cells and one Human Fetal-Derived Stem Cells.

After harvesting precursors of BM the patient was placed in a right lateral position with a 30° to 45° inclination from the horizontal plane of the right hemithorax.

Video-assisted surgery includes positioning of three 10mm trocars in the 3rd, 5th and 7th intercostal spaces: One for the camera and the rest for the instruments. We used a double-lumen oro-tracheal tube for this procedure, and the trocars were introduced once the left lung had been isolated. Subsequently, the camera was inserted in the 7th space (Figure 2).

Exploration began by opening the pericardium after observing the position of the frenic nerve. Injection in the selected areas was performed, guided by the preoperative echocardiogram and avoiding intraventricular and intracoronary injection. We delivered aliquots of 1cc separated by 2 to 3cm and 3 to 5mm in depth with a 25G needle with an average of 25 injections in the ABMSCs patients (Figure 3). Only 15 injections were made in the anterolateral wall in the patient who received HFDSCs (S.B., female, 48).

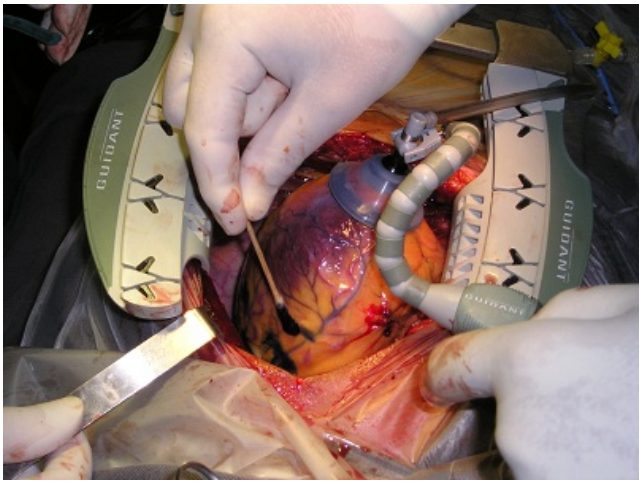

Figure 1: Sternotomy blue marker for the 80 injections

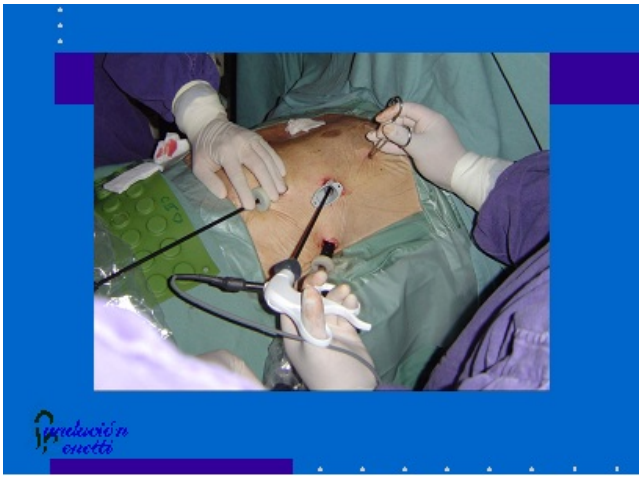

Figure 2: Minimally invasive approach for stem cells implantation

The procedure was completed when trocars were withdrawn and the thoracic tube was put into place. Finally, the four patients were extubated and release the OR.

Follow-up was conducted through clinical examination, EKG, X-ray films and echocardiogram at 48 months' time after surgery. Results were analyzed by independent cardiologists, not involved in this study.

The student test statistical analysis (SPSS program) was carried out showing $p<0.05$ which was considered significant.

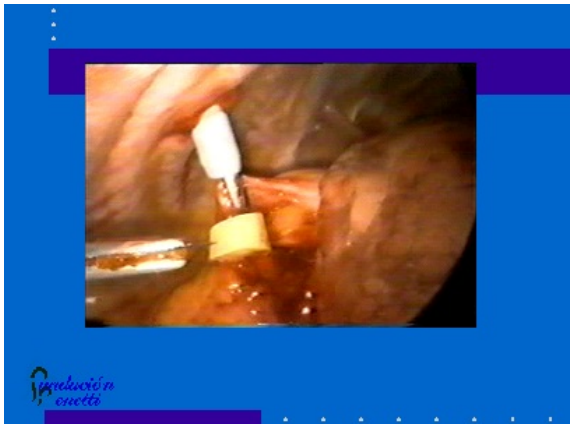

Figure 3: Stem cells injection in the heart by minimally invasive approach

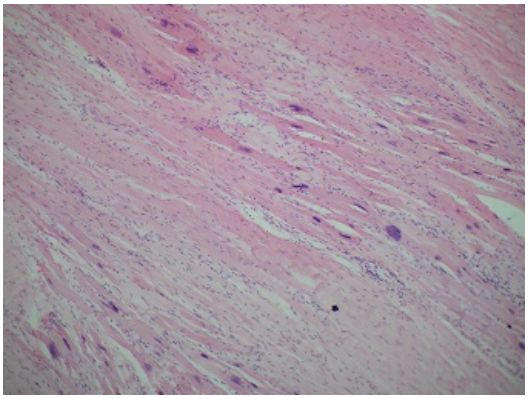

Figure 4: The heart autopsy showed nests of cardiomyocytes among the fibrotic tissue.

Results

Human fetal-derived stem cells

Six female and four male patients (age range, 47-77 average 58) met the inclusion criteria and participated in the study. Nine of those received HFDSCs by sternotomy and one minimally invasive left approach. There was no surgery or postsurgery mortality.

One male patient (U.J., 69) experienced a single transient in-surgery ventricular fibrillation during the procedure but before receiving injections; the ventricular fibrillation was terminated by electrical cardioversion.

One man (M.J., 66) and one woman (V.M., 77) required temporary pacemakers post-surgery because of 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 after 3 weeks, which was solved spontaneously. He was later excluded from the trial for noncompliance (he abandoned his controls), and he ultimately died after 5 months. The heart autopsy showed nests of cardiomyocytes in the fibrotic tissue (Figure 4), but it was not possible to determine whether they were new growing myocardium tissue or remaining native fibers.

One female patient died of diabetes complication after 18 months.

Another female patient died suddenly of severe gastroenteritis complications after 8 months. This patient suffered several episodes of this illness and were not related of the treatment with stem cells.

The female patient who underwent her procedure via minimally invasive died from HF caused by mitral insufficiency after 12 months. She had a minimal mitral insufficient previous to the injections. Probably because she improved the contractility of the left ventricle the mitral insufficiency was aggravated and she had refused a mitral valve operation.

The HFDSCs patients who provided 40 months of follow-up data showed improvements both clinically and in imaging studies. With regard to the imaging studies, we noted an increased wall thickness, both eccentric and concentric.

Patients improved in association with increased contractility in these regions. Compared with baseline assessments, we noted other improvements: The mean (±SD) NYHA class decreased from III/IV ± 0.5 to I/III±0.5 (P=.001); the mean EF increased 31%, from 26.6% ±4.0% to 34.8%±7.2% (P=.005); performance in the ETT increased 291.3%, from 4.25 minutes to 16.63 minutes (128.9% in metabolic equivalents, 2.45 to 5.63) (P<.0001); the mean LVEDD decreased 15%, from 6.85±0.6cm to 5.80±0.58cm (P<.001); mean performance in the 6-minute walk test increased 43.2%, from 251±113.1 seconds to 360±0 seconds (P=.01); the mean distance increased 64.4%, from 284.4±144.9m to 468.2±89.8m (P=.004); and the mean result in the Minnesota congestive HF test decreased from 71±27.3 to 6±5.9 (P<.001) The Kaplan-Maier probability of survival test after 40 months was 66% .

Rejection was not observed. These patients not develop malignant nodules or cancer at all in the follow-up.

Autologous bone marrow stem cells

Three patients, two males and one woman (age range 62-71 average 67.6 years), from the heart Failure Unit, complied with the inclusion criteria and received autologous stem cells by minimally invasive left approach. All patients were extubated in the OR. The pleural tube was withdrawn after 24hrs and they were discharged after an average of 2.26 days. No major complications such as stroke, MI, significant post-surgical arrhythmias, infection of the surgical site, or renal failure were observed, and no deaths occurred. They received an average of 54X106/mL CD_3^{4+} cells. Despite the patientients' baseline NYHA functional class III/IV, this medical treatment proved to be significant at six months after receiving the stem cell treatment the average functional class value was I/III, showing a marked clinical improvement. This significant difference ($p<0.05$), represents a very positive change in the patients' quality of life. After 6 months all of them remained in functional class I/II. Baseline values of LVEF markedly decreased, ranging between 25 to 30%. Significant differences were detected after 6 months with an increase of 38, 40 and 46%. There was a similar change in ventricular diameters: after 6

months the LVESV went from 50mm to 42mm ($p<0.05$). After 24 months, two of the patients still maintained this improvement, while the third patient returned to the earlier values after suffering from pneumonia. After 100 and 110 months the actual average EF was 28 and 30%. The third patient died of sudden death after 124 months after the implantation. We cannot account for the cause of this sudden death because we could not do a detailed long-term follow-up of this patient.

After 150 months two patients were still alive and both received a Resynchronization Therapy. It is impossible to affirm if the deterioration of the ventricle was related to the surgical technique or the type of cells injected after this long period of follow-up.

Discussion

Stem cell treatments depend not only on the type of cells used but also on the way of approaching the target organ or tissue. It is known that intravascular injections of cells rapidly clear from the target vessel. On the other hand it is also well known that the direct injection into the myocardium provides better engagement within the cardiac muscle. Therefore, either in the case of HFDSC or ABMSC we focused on direct approaches.

When we had to deal with different stimuli, such as parietal stress or direct myocardial injury leading to hemodynamic overload, the heart responds with hypertrophy, capable of initially compensating loss of function. Later on, and for a long sub-clinical period, progressive dilatation continues to be compensated by varying degrees of hypertrophy. At the final stage, as described by Meerson et al. [19], dilatation exceeds hypertrophy and changes in cellular organization appear, such as: 1) Myofibrillar lysis; 2) Increase in lysosomes; 3) Distortion of the sarcoplasmic reticulum; 4) substitution of myocardial cells by fibrous tissue.

Simultaneously, capillary density and contractile reserve decrease, and diffuse myocytic necrosis is a feature of both idiopathic and ischemic dilated cardiomyopathy [20,21].

Therefore, idiopathic dilated cardiomyopathy can be described from a pathological point of view [22] as a dilated heart with hypertrophied walls: the macroscopical dilatation exceeds the hypertrophy. Microscopically the heart appears invaded by areas of interstitial and perivascular fibrosis, adjacent to necrotic areas and myocytes which may be atrophic or hypertrophic, with loss of the extracellular matrix. Cellular therapy in these patients is directed to restore and repopulate the myocardium, and thus recover the lost function by delivering cells that are able to differentiate into myocardial cells.

The use of mesenchymal or stromal cells as precursors of non-hematopoietic tissues was attempted for the first time by the German pathologist Conheim in 1867 [23]. It was later shown in tissue cultures that they were capable of forming diverse tissues, such as bone, cartilage, muscle, ligaments, tendons, etc [23,24], and of intervening in tissue repair [23].

A very interesting study by Makino S et al. [25] shows that stromal stem cells treated with 5 Azacytidine and injected with cardiomyocytes, form interconnections between cells and micro-tubuli one week later. After 2 weeks, they started beating and this contraction became synchronous, after three weeks they developed natriuretic peptide and were stained with antibodies against actin and myosin, and presented a potential action of cardiac cells [25].

The trans-differentiation into the cardiac phenotype requires an adequate microenvironment. Dependence on cellular interconnection by generating cardiac transcription factors (GATA-4 and myocytic factor-2 was also observed). When cocultured with myocytes, Bone Marrow Stem Cells (BMSCs) can transdifferentiate into cells with a cardiac phenotype. Differentiated myocytes express cardiac transcription factors GATA-4 and myocyte enhancer factor-2. The transdifferentiation processes rely on intercellular communication of BMSCs with myocytes [26]. This also was confirmed by other researchers, stressing the importance of cardiomyocyte intercellular integration with trans-differentiated cells [27]. Among the various cell types studied, stromal stem cells were shown to present the capacity for differentiation into muscular and vascular cells, and for producing neo-vascularization. The first reported case of BM cells applied to cardiomyoplasty was by Weisel and Lee of Toronto University in 1999 [28].

This differentiation into myogenic lineage with development of actin, myosin and tropomyosin was proved, as well as the presence of Conectin 43, a protein responsible of cellular inter-connection.

Several stem cell mechanisms that work improving cardiac function have been proposed:

Fusion and trans differentiation: Fusion with local cells has been ruled out; and the attractive concept of stem cells trans-differentiating and crossing the barrier has still to be demonstrated. Perhaps the correct anatomical mechanism will be clarified when cell therapy can be used as a bridge for heart transplantation and biopsies can be performed.

Cardiac stem cell mobilization: Another theory proposes that the implanted cells mobilize specific cardiac stem cells lodged in the muscle that are capable of cardiac regeneration.

Angiogenesis: The implanted cells induce significant angiogenesis; according to some, this would be the main mechanism involved.

Extracellular matrix: In heart failure there is a net loss of myocytes plus a loss of matrix architecture, which leads to dilatation. The implanted cells could stabilize the latter, preventing dilatation and balancing generation/degradation enzymes. This is the mechanism thought to cause Chagas disease [29].

Clinical application of this treatment was started in 2000, and cases have increased throughout the world. Our study on cases of idiopathic dilated cardiomyopathy shows that it is a feasible procedure, and it has no surgical or immediately postoperative mortality.

This technique has shown extremely good results in prospective, randomized studies in ischemic patients, taken to the OR for myocardial revascularization: Marked improvement was observed in ventricular ejection fraction and functional class in those receiving stem cells compared with those submitted to revascularization alone [30]. Clinical benefits were evident in the improvement of functional class, and no negative effects could be seen.

Patients receiving autologous cell therapy obtained a significant increase in LV ejection fraction immediately and improved the clinical condition.

We recognize that the relatively small number of patients may represent a significant limitation to this study. These initial findings suggest, however, that HFDSC transplantation improves cardiac function in HF patients suffering from idiopatic cardiomyopathy. No

rejection reactions or developed of malignant nodules has been seen after 40 months [31].

We believe that the sustained effect of HFDSC therapy indicates that it offers another possibility for treating patients with advanced HF and represents a new approach that could be used before other major surgical treatments, including heart transplantation, by having them available "on the shelf" thereby avoiding the time-consuming procedures of autologous bone marrow harvesting and processing. Irrespective of the improvement seen in this study, it is still premature to determine accurately the mechanism of action, indications, and doses. Therefore, it is imperative and extremely important that further research needs to be done.

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