Effective combined off-pump surgical treatment and autologous bone marrow cell transplantation: a new alternative for patients with end-stage ischemic cardiomyopathy (Prapas' procedure)

Otolog kök hücre transplantasyonu ve pompasız cerrahi tedavinin etkili birlikteliği: Terminal iskemik kardiyomiyopatili hastalar için yeni bir alternatif (Prapas prosedürü)

Sotirios Prapas, Dimitrios Protogeros, Fotini Danou, Chrysanthi Trikka**, Ioannis Panagiotopoulos, Helen Chandrinou****

From Departments of Cardiac Surgery, *Anesthesia, **Cardiology, and *** Immunohematology and Transfusion Medicine, Henry Dunant Hospital, Athens, Greece

ABSTRACT

Objective: To propose an alternative method combined off-pump treatment of end-stage ischemic cardiomyopathy consisting of revascularization of ischemic areas, external reshaping of the left ventricle (LV) in order to restore near normal geometry and autologous bone marrow-derived mononuclear cell (BM-MNC) implantation.

Methods: Forty- seven patients (mean age 58±8.9 years) underwent the above procedure. All patients were NYHA III-IV and four were transplantation candidates. They underwent standard laboratory evaluation, transthoracic echocardiography, dipyridamole thallium scintigraphy (DTS) and cardiac magnetic resonance imaging preoperatively and at 3rd, 6th and 12th months postoperatively. After revascularization and external LV reshaping, BM-MNCs were injected into predetermined peri-infarct areas.

Results: Forty-five patients survived during a follow up period of 3-37 months. Ejection fraction improved from 21.7±7.4% to 30.6±6.9%, 36.5±4.3% and 37.7±4.2% at 3rd, 6th and 12th months, respectively. Left ventricular end-diastolic diameter was reduced from 66.1±4.9 mm to 62.6±3.9 mm, 60.5±2.9 mm and 59.3±4.2 mm respectively. Previously non-viable areas on DTS were found to contain viable tissue and MRI showed hypokinesia in previously akinetic areas. NYHA class improved to I-II. No significant arrhythmias were noted during the follow-up period. One patient died due to low cardiac output and one patient died due to septic shock.

Conclusions: Combined off-pump surgical treatment and autologous bone-marrow mononuclear cell transplantation for end-stage ischemic cardiomyopathy is safe and feasible and appears to improve the patients' functional status.

(Anadolu Kardiyol Derg 2008; 8: Suppl 2; 101-7)

Key words: Ischemic cardiomyopathy, off-pump surgery, cell transplantation

ÖZET

Amaç: Bu çalışma son safhada iskemik kardiyomiyopatisi olan hastalar için iskemik alanların revaskülarizasyonu, normal geometriye dönüştürülmesi amacı ile sol ventrikülün (LV) dışardan yeniden şekillendirmesi ve otolog mononükleer kök hücrelerin (BM MNC) implantasyonuna dayanan kombine alternatif bir tedavi yöntemi geliştirmek amacı ile yapılmış.

Yöntemler: Ameliyat toplam 47 hastaya uygulandı (ortalama yaş, 58±8. 9 yıl). Bütün hastalar, evre III ve IV NYHA sınıfında idi ve 4'ü transplantasyon adayı idi. Tüm hastalara standart laboratuvar değerlendirme, transtorasik ekokardiyografi, dipiridamol talyum sintigrafisi (DTS) ve preoperatif, postoperatif 3., 6., 12. aylarda kardiyak manyetik rezonans görüntülemesi (MRI) yapıldı.

Otolog mononükleer kök hücreleri, revaskülarizasyon ve LV yeniden şekillendirme işlemi sonrasında önceden belirlenen peri-infarkt alanlarına enjekte edildi.

Bulgular: Üç ile otuz yedi ay izlem döneminin sonunda 47 hasta hayatta kaldı. Ejeksiyon fraksiyonu 3., 6. ve 12. ayda %21.7±7'den sırası ile %30.6±6'ya, %36.5±4.3'e ve %37.7±4.2'ye kadar yükseldi. Sol ventrikül diyastol sonu çapı aynı dönemlerde 66.1±4.9 mm'den 62.6±3.9 mm, 60.5±2.9 mm ve 59.3±4.2 mm'ye kadar azaldı. İzlem sonunda DTS'de önceden canlı olmayan alanlarda canlı dokular tespit edildi ve MRI'da, önceden akinetik olan alanlarda hypokinezi görüldü. NYHA sınıfı, evre I- II'ye azaldı. İzlem sırasında hiçbir önemli aritmi tespit edilmedi. Bir hasta, düşük kardiyak debi sendromu ve bir hasta septik şok nedeni ile kaybedildi.

Sonuç: Kombine pompa-dışı cerrahi tedavi ve otolog mononükleer kök hücre transplantasyonu son safhada iskemik kardiyomiyopatili hastalar için güvenilir, uygulanabilir ve hastaların fonksiyonel durumunu iyileştiren bir yöntemdir. *(Anadolu Kardiyol Derg 2008; 8: Suppl 2; 101-7)*

Anahtar kelimeler: İskemik kardiyomiyopati, "off-pump" cerrahi, hücre transplantasyonu

Address for Correspondence/Yazışma Adresi: Prof. Sotirios Prapas, 107 Mesoghion Avenue, 11526 Athens, Greece
Phone: +302106972877 Fax: +302106972735 E-mail: sprapas@dunanthospi.gr

Introduction

Congestive heart failure (CHF) is a major cause of death and disability in western societies. It affects 6-10% of people over the age of 65 and its incidence is increasing, leading to an increased number of hospitalizations and health care costs (1, 2). About two thirds of cases are due to ischemic heart disease, where myocardial mass is lost after an acute infarction. The remodeling process which follows, although useful initially, eventually leads to chamber dilatation, loss of normal geometry and further compromise of heart function (3).

The mainstay of CHF treatment is conservative. This may lead to amelioration of symptoms but does not seem to offer long term benefits on survival (4). Surgical options are heart transplantation, ventricular assist devices (VADs) or, in selected patients, revascularization combined with ventricular aneurysmectomy. Heart transplantation is limited by the lack of donors and requires chronic immunosuppression, which is accompanied by serious risks. Ventricular assist devices are most commonly used as a bridge to transplantation and their usefulness is limited by potential complications such as bleeding, infection, thromboembolic episodes etc. Additionally, they have a significant cost, a limited life and the quality of the patients' life is usually compromised (4, 5). Revascularization with aneurysmectomy may be applied to patients with large aneurysms. This procedure requires the use of extracorporeal circulation, which is associated with significant morbidity, particularly in such severely ill patients. In addition, large, well-defined aneurysms are rarely seen today due to the evolution of interventional cardiology and the improvement of treatment after acute ischemic events (6, 7). More commonly, a combination of akinetic and/or dyskinetic areas is seen, with variable amounts of viable myocardium in between (8).

Recent stem cell research has raised hopes for myocardial repair or regeneration. Studies in animals (9-15) and humans (16-23) suggest that a number of cells, isolated from skeletal muscle, bone-marrow or peripheral blood may engraft into the scarred myocardium and the surrounding areas resulting in functional improvement via a yet to be defined mechanism. Angiogenesis, vasculogenesis, differentiation into muscle or myocardial cells and more complex paracrine mechanisms have been suggested as potential pathways (15, 24, 25).

However, end-stage ischemic cardiomyopathy is a complex syndrome and it is unlikely that a single treatment, even cell therapy, would prove to be a "universal" cure. Similar to the multimodal pharmacological treatment of CHF, a combined surgical approach seems more reasonable.

Based on this rationale, we treated patients with end-stage ischemic cardiomyopathy with a combination of surgical revascularization, external reshaping of the LV and implantation of autologous bone marrow-derived mononuclear cells on an off-pump basis, in order to investigate the safety and efficacy of this combined technique.

Methods

Between July 2005 and August 2008, 47 patients underwent the combined surgical treatment after informed consent. The inclusion criteria were diagnosis of HF of ischemic etiology, NYHA class III or IV, ejection fraction (EF) ≤35%, left ventricular end-diastolic diameter (LVEDD) ≥55 mm, large areas of irreversible ischemia and at least one target suitable for revascularization. Exclusion criteria were chronic renal dysfunction (serum creatinine >2.0 mg/dl), hepatic failure, hematologic disease, history of malignancy, history of cerebrovascular disease, diffuse vascular disease, history of severe arrhythmias, severe primary lung disease, body surface area >2.0 m^2, age <18 or >75 years and insulin dependent diabetes mellitus. Preoperative evaluation included NYHA class assessment, coronary angiography, hematologic assessment, pulmonary function tests and standard blood tests. Transthoracic echocardiography was performed by a single cardiologist, blinded to the procedure, in order to measure EF and LVEDD. Cardiac magnetic resonance imaging (MRI) and/or dipyridamole thallium scanning were used to identify and map scarred areas of the left ventricle in detail. All tests were repeated at 3^{rd}, 6^{th} and 12^{th} months.

Operative technique

Intraaortic balloon pump (IABP) was inserted in all patients immediately before surgery.

In 15 patients, after induction of anesthesia and institution of invasive monitoring, the patient was positioned appropriately and bilateral posterior iliac crest aspiration was performed under strictly sterile conditions. The bone marrow was aspirated with 10 ml syringes rinsed with a solution containing 1000 ml normal saline and 25000 units of heparin to avoid clotting. The contents of each syringe were then transferred in the collection bag of the Bone Marrow Collection Kit (R4R2107; Fenwall, Baxter), where 40 ml of anticoagulant ACD-A (Acid Citrate Dextrose-Adenine solution, Macoflex) was added to obtain a final volume of approximately 450ml of bone marrow, aiming at isolating a large number of cells (10^9). In the cellular and molecular therapy, the bone marrow was filtered by gravity through a series of successively smaller diameter mesh filters (500μm and 200μm) to eliminate bone spicules, fat and cellular debris and then was collected in a sterile plastic transfer bag. The bone marrow mononuclear cells (BM-MNCs) were isolated using the Bone Marrow Processing (BMP) procedure, sorting the cells on a Spectra cell separator (777006-300; Cobe Spectra Apheresis System, Gabro BCT). During this procedure, anticoagulated bone marrow enters the inlet chamber through the inlet tube; as it flows through the channel, it is separated into three layers: the red blood cells on the outer layer, the buffy coat containing the white blood cells in the middle and the platelet rich plasma in the inner layer. Maintaining the red blood cell/plasma interface in a constant position, the white blood cells (WBC) are drawn from the channel through the WBC collect tube, and the MNCs are concentrated to a volume of 80 ml. The cellular product is diluted by an equal volume of Dulbecco's phosphate-buffered saline (PBS, calcium and magnesium free, GIBCO) with 5% human serum albumin. The cell suspension is placed over HISTOPAQUE-1077 (Sigma-Aldrich) 3:1 and the remaining red blood cells are removed by density-gradient

centrifugation at 400G for 30 minutes. The upper layer is aspirated, leaving the mononuclear cell layer at the inter-phase. The inter-phase cells are transferred to a new conical tube and exhaustively washed with PBS containing 5% human serum albumin by centrifugation at 1200G for 20 minutes twice. The supernatant is completely removed and the cell pellet is re-suspended in saline solution with 5% human serum albumin for injection at a final volume of 30 ml.

Bone marrow cells in 32 patients were isolated using the BMAC system (Harvest Technologies Corporation, Plymouth, MA) according to the instructions of the manufacturer Approximately 60 ml of bone marrow was collected from posterior iliac crest of each patient. The system uses a dual chamber disposable. The first chamber contains a floating shelf of a specific density into which the heavy red blood cells are separated from the nucleated cells, platelets and plasma during the initial centrifugation phase. The cellular elements and plasma are automatically decanted into the second chamber and concentrated by centrifugation. A portion of the supernatant plasma is removed and the cellular elements are resuspended in saline solution with 5% human serum albumin for injection at a final volume of 30ml.

A small fraction of the cell suspension is reserved for cell counting, cultures and viability testing with trypan blue exclusion. Cell viability was shown to be consistently above 90% (95±4%) thus assuring the quality of the cell suspension. Bacterial and fungal cultures of the clinically used cell preparations were performed and proved negative. An average of 1.5±0.8 x 10^9 BM-MNCs were isolated per patient (range 0.85-3.03 x10^9).

Meanwhile, the operation was performed through a median sternotomy. The first step was to revascularize the identified coronary targets using arterial grafts whenever possible. The aorta non-touch technique was applied. Both internal mammary arteries were harvested in a skeletonized fashion and preconstructed conduits (Y, T, Π, I) (Fig 1), using the free right internal mammary, radial or saphenous vein, were prepared according to the required grafts (26, 27).

After completing the revascularization, the external reshaping was performed off-pump. The heart was positioned appropriately and the dilated, akinetic/dyskinetic areas were defined. Superficial interrupted mattress sutures reinforced with Teflon felt or pericardial strips were used to plicate these areas, taking care not to injure adjacent or underlying vessels (Fig. 2-5). Before tying, the sutures were put under tension simultaneously after temporarily reducing the LV preload, using a small nitroglycerine bolus and the head-up position when needed, so that tension was evenly distributed along the suture lines (28).

The BM-MNCs were then injected into the peri-infarct areas of the heart, as defined by preoperative testing (echocardiography, MRI, scintigraphy), through a 24G butterfly needle which was inserted at a 45^0 angle into the myocardium (Fig. 6). Approximately 15-30 punctures were performed, injecting 0.5 ml at each puncture.

Statistical analysis: Data are presented as mean±SD.

Results

Forty-four patients were male and the mean age of all patients was 58±8.9 years. Thirty-seven patients were in NYHA class III and

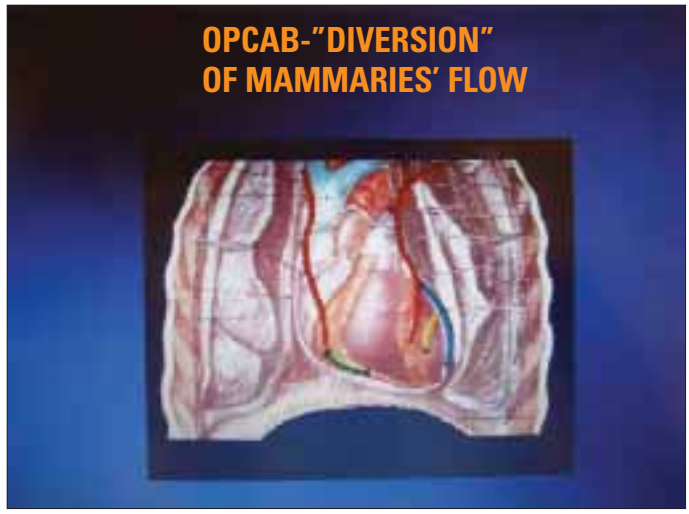

Figure 1. Π**- Circuit**

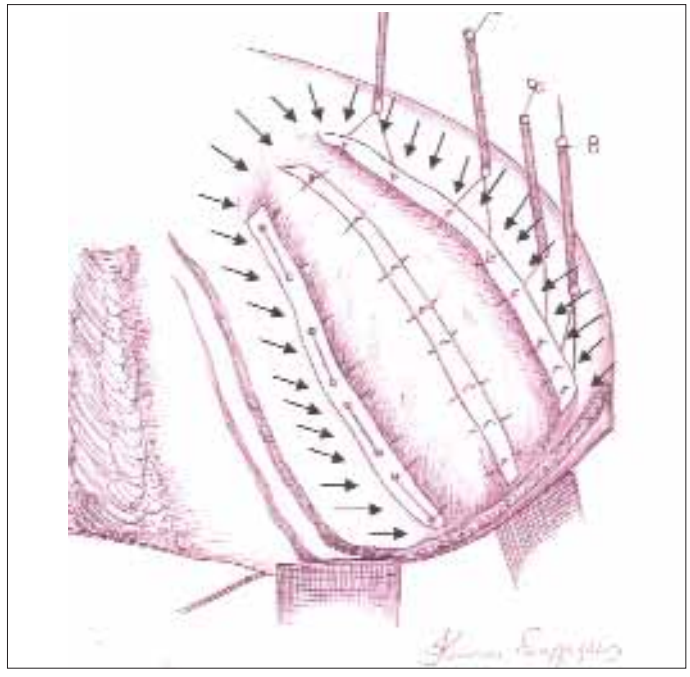

Figure 2. Schematic representation of external LV reshaping (arrows indicate areas around the infarct injected with cells)
LV-left ventricular

10 in class IV (mean value 3.4). The mean preoperative EF was 21.7±7.4% and the LVEDD was 66.1±4.2 mm.

At a follow-up period of 3-37 months only two patients died. The first one died due to low cardiac output and the second one due to septic shock. All the other patients are alive and their clinical assessment reveals a significant improvement in their symptoms and quality of life (average NYHA class 2.4, 1.5 and 1.5 at 3rd, 6th and 12th months respectively). Ejection fraction improved to 30.6±6.9%, 36.5±4.3% and 37.7±4.2% at 3rd , 6th and 12th months, while LVEDD was reduced to 62.6±3.9 mm, 60.5±2.9 mm and 59.3±4.2 mm respectively.

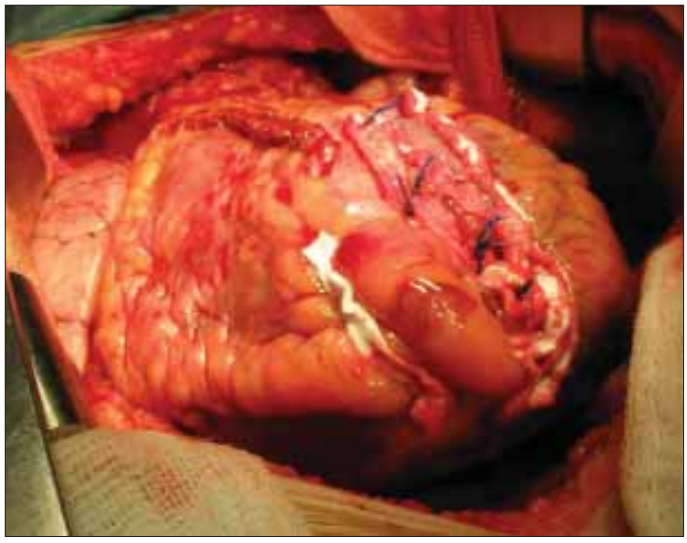

Figure 3. Anteroapical plication

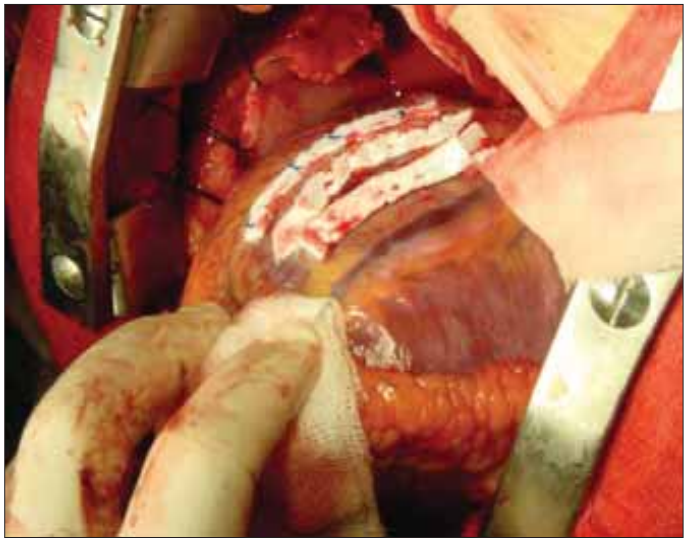

Figure 4. Plication of the inferolateral wall

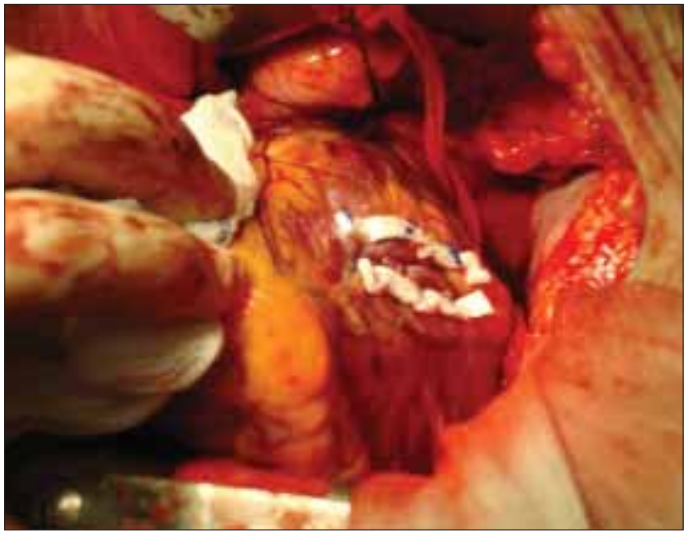

Figure 5. Plication of the inferior wall

The changes in EF and LVEDD over time are illustrated in Figures 7 and 8 respectively.

For logistic reasons, only thirty-two patients were tested with cardiac MRI and thirty six had a dipyridamole thallium scan. The areas of akinesia/ dyskinesia and the measured EF and LVEDD were in agreement with the echocardiography findings. At six months and one year, apart from the improvement of global EF and LVEDD, thallium scanning or MRI revealed hypokinesia or reduced distribution of radioopaque contrast in areas where previously akinesia or lack of distribution were observed.

No severe adverse effects were noted. There was no bleeding from the iliac crest puncture sites. Three patients developed supraventricular arrhythmia (atrial fibrillation or atrial extrasystoles) in the first 24 hours postoperatively, which were treated successfully with amiodarone. Continuous rhythm monitoring throughout hospitalization and Holter examination 3 months after discharge revealed no further arrhythmias.

Twelve patients did not require external reshaping because no clearly defined akinetic/dyskinetic area was identified. One of them had significant mitral regurgitation due to LV dilatation and required mitral valve repair. In this patient, stem cells were implanted after weaning from bypass and full reversal of heparin. Patient No 9 was the most challenging as he had suffered an acute anterolateral myocardial infarction 6 weeks before surgery. He went into acute heart failure (EF 10%), complicated by pulmonary infection and soon developed acute renal failure (creatinine up to 5.2 mg/dl). Therefore, the option of LVAD was excluded and the patient remained intubated and supported by IABP in alternating femoral arteries. Although he was undergoing hemofiltration, he was not excluded from our treatment group because it was felt that the renal failure was prerenal, of reset onset and potentially reversible. Considering the lack of options, we decided to wait for 6 weeks until the infarcted area healed and became less friable in order to operate. He underwent a single bypass to the left anterior descending artery, external reshaping of the anterior wall and the apex and was injected with (number) of BM-MNC in the peri-infarct areas of the anterior and anterolateral wall. The procedure was successful and the patient was discharged with normal renal function and in a good clinical state.

Discussion

Our initial experience with the combined surgical/cellular approach for end-stage ischemic heart failure shows that it is feasible, safe and seems to improve patients' cardiac function and quality of life.

We chose to apply this technique in patients with severe ischemic cardiomyopathy, as these patients have no or very limited options and, once they are diagnosed with stage C or D CHF, their life expectancy averages 1.7 years (American Heart Association, 2002). The possibility for heart transplantation in Greece is remote, as only 3.17 transplantations are performed each year due to lack of donors (29). It is important to note that 4 of our patients were transplantation candidates.

It is difficult to deduce which one of the three aspects of the intervention (revascularization, external reshaping or stem cell transplantation) was the most effective. Yet, we felt that this multimodal approach would be more beneficial considering the complexity and severity of the patients' condition. In a recent review, Angelini et al (30) pointed out that cell treatment would be unlikely to yield a viable and functional myocardium if the normal blood supply were not also restored. The efficacy of stem cell therapy is still under investigation and the mechanism through which it works is unclear (24). Yet, studies so far that show beneficial effects cannot be overlooked. It appears that it takes at least three months for such effects to become significant (20, 22, 31). Therefore, it seems reasonable to give the myocardium any assistance possible by providing optimal blood supply to the diseased coronaries. Additionally, it is possible that in advanced CHF, where the LV is severely dilated and dyskinetic, even myocardial regeneration may not be enough to adequately reverse the remodeling process and achieve near-normalization of the LV size and shape (30). The external reshaping procedure that we describe may assist in this direction. Depending on the extent to which it is applied, it can either prevent the progress of chamber dilatation or even reduce LVEDD and improve LV geometry as shown by echocardiography. Our group has published the application of this technique in 57 patients with good results and no adverse effects (28). A significant advantage is that it can be performed off-pump. In contrast to traditional surgical techniques (32-34) it does not involve excision of any LV segments, which would be significantly more traumatic and could lead to loss of viable segments of myocardium, while disturbing the continuity of the cardiac muscle (30).

Performing the whole procedure off-pump is of great importance. Adding the risks of cardiopulmonary bypass, cardioplegic arrest and aortic manipulations to this already severely compromised patient population would most likely increase significantly the perioperative morbidity and mortality.

We were obliged to use IABP in all patients in order to be able to manipulate the heart as needed without compromising cardiac output and perfusion pressures. In 18 cases, the addition of levosimendan, a new inodilator, which acts through calcium sensitization was used in addition to IABP. This agent was chosen over traditional inotropes such as dobutamine or adrenaline as it is less arrhythmogenic, it does not significantly increase myocardial oxygen demand and has a prolonged effect after discontinuation (35). No patient suffered any complications such as life-threatening or persistent arrhythmias, cerebrovascular accident, renal failure etc.

Measurements of EF and LVEDD were based on echocardiography which remains the cornerstone of diagnosis and follow-up of heart failure (4, 36, 37). It is a simple and inexpensive test, which is most widely applied. A single, blinded cardiologist performed the examination in all patients to avoid interobserver variability and bias. Although there is increasing literature supporting the use of MRI, which offers more detailed

Figure 7. Plot of EF mean values ± SD and ± SE. (Values analyzed from 37 patients with full data)

LVEF - left ventricular ejection fraction

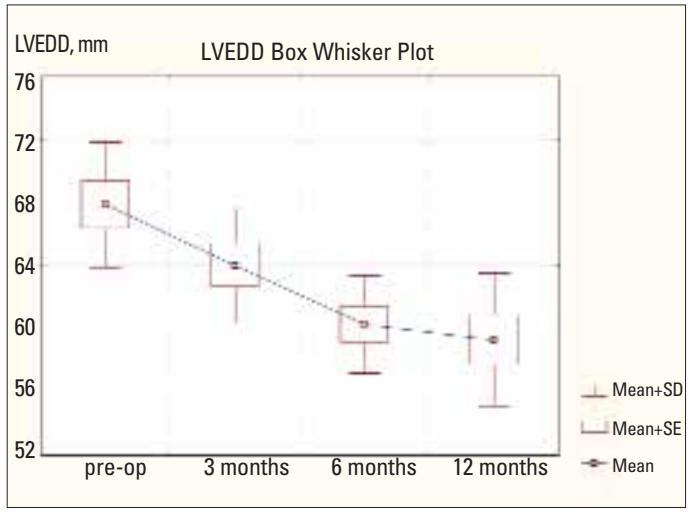

Figure 8. Plot of LVEDD mean values ± SD and ± SE. (Values analyzed from 37 patients with full data)

LVEDD - left ventricular end-diastolic dimension

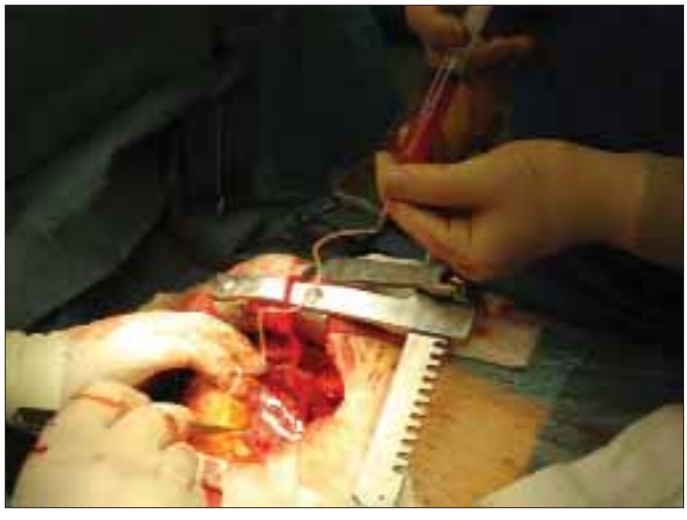

Figure 6. Stem cells implantation

information about the morphology, viability and motion of myocardial segments (38-41), this test is not as widely available and its cost is significant. We were able to obtain cardiac MRI in thirty-two patients. Its findings paralleled those of echocardiography.

In six patients, we used dipyridamole thallium scanning to determine viable and non-viable areas. It was interesting to find some distribution of radioopaque contrast, during the 6^{th} and 12^{th} month follow-up, in areas that were preoperatively identified as non-viable. These areas were not revascularized, as no target could be identified and received only stem cell implantation. This might suggest cell-mediated neovascularization and/or regeneration of these areas consistent with the growing consensus that bone-marrow cells are angiogenic.

There are many reasons why we selected bone marrow-derived mononuclear cells: first, they comprise a mixed population of multipotent cells (haematopoietic, endothelial progenitor and mesenchymal or stromal cells) (5, 42). They are autologous, therefore there are no ethical concerns regarding their use and they are devoid of immunologic reactions. Their cardiac application has not been associated with arrhythmias (16, 18, 20, 23, 43). They can be harvested on the day of operation and prepared within 3-4 hours at a relatively low cost. Until there is clearer evidence that a certain subpopulation of these cells or another cell type is more effective, it appears reasonable to apply the simplest and most cost-effective option.

It has been suggested that the number of transplanted cells is quite important for the effectiveness of cell therapy, while there are questions about the number of cells that are retained after application (24). Our dose was on the order of 10^9, which to the best of our knowledge has not been used in other studies. To achieve such numbers we had to collect 400 ml of bone marrow, which was not associated with any adverse effects.

Our exclusion criteria were extensive compared to other studies. The reason was that we chose to exclude severe co-morbidities, which could complicate further the condition of the patients. It is likely that some of these criteria (e.g. diabetes mellitus, peripheral vascular disease, primary lung disease) will become only relative contraindications as we gain more experience.

We are aware of the limitations of a non-randomized, non-controlled study. Yet we could not find an ethically plausible reason to operate on such a patient population without using all three components of our technique simply in order to form a comparable control group. They are designed to complement each other and in patients with end-stage CHF and no further options we felt that we should try to offer a maximum potential benefit. We believe that our encouraging results could be a foundation on which a larger controlled, randomized study could be designed, aiming perhaps at less severely ill patients, although preliminary data from other groups suggest that the most ill patients are those with the greatest response potential (44, 45).

Conclusions

In conclusion, our initial experience shows that patients with end-stage heart failure of ischemic etiology may be treated safely and effectively by a combined surgical procedure comprising of revascularization, external reshaping of the LV and autologous bone marrow-derived mononuclear cell transplantation on an off-pump basis. Its application is simple as it does not require additional skills, when performed by a surgeon experienced in off-pump coronary surgery, it has a lower cost than other surgical options and a low complication rate. Furthermore, it does not exclude the patients from alternative treatments in the future. Perhaps the most important finding is that results are maintained for more than three years, leading to a significant improvement of the patients' cardiac function and quality of life.

References

1. American Heart Association. Heart Disease and Stroke Statistics-2005 Update. Dallas, Texas: American Heart Association, 2005.
2. Berry C, Murdoch DR, McMurray JJ. Economics of chronic heart failure. Eur J Heart Fail 2001; 3: 283-91.
3. Cohn JN. Structural basis for heart failure. Ventricular remodelling and its pharmacological inhibition. Circulation 1995; 91: 2504-7.
4. Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS, et al. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary. A report of the American College of Cardiology/ American Heart Association task force on practice guidelines (Committee to revise the 1995 guidelines for the evaluation and management of heart failure). J Am Coll Cardiol 2001; 38: 2101-13.
5. Rosenstrauch D, Poglajen G, Zidar N, Gregoric ID. Stem cell therapy for ischemic heart failure. Tex Heart Inst J 2005; 32: 339-47.
6. Cooley DA, Henly WS, Amad KH, Chapman DW. Ventricular aneurysm following myocardial infarction: Results of surgical treatment. Ann Surg 1959:150; 595-612.
7. Cheng TO. Incidence of ventricular aneurysm in coronary artery disease: An angiographic appraisal. Am J Med 1971: 50; 340-55.
8. Buckberg GD. Defining the relationship between akinesia and dyskinesia and the course of lest ventricular failure after anterior infarction and reversal of remodelling to restoration. J Thorac Cardiovasc Surg 1998; 116: 47-9.
9. 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.
10. Chiu RC, Zibaitis A, Kao RL. Cellular cardiomyoplasty: Myocardial regeneration with satellite cell implantation. Ann Thorac Surg 1995; 60: 12-8.
11. Li RK, Jia ZQ, Weisel RD, Mickle DA, Zhang J, Mohabeer MK, et al. Cardiomyocyte transplantation improves heart function. Ann Thorac Surg 1996; 62: 654-60.
12. Scorsin M, Hagege AA, Marotte F, Mirochnik N, Copin H, Barnoux M, et al. Does transplantation of cadiomyocytes improve function of infracted myocardium? Circulation 1997; 96 (II Suppl): 188-93.
13. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate myocardium. Nature 2001; 410: 701-5.
14. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001; 107: 1395-402.
15. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, et al. Implantation of bone marrow cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands and cytokines. Circulation 2001; 104: 1046-52.

16. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT, et al. Transendocardial, autologous bone marrow cell transplantation for severe chronic ischemic heart failure. Circulation 2003; 107: 2294 -302 .
17. Siminiak T, Fiszer D, Jerzykowska O, Grygielska B, Rozwadowska N, Kaımucki P, et al. Percutaneous trans-coronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: the POZNAN trial. Eur Heart J 2005; 26: 1188-95.
18. Patel AN, Geffner L, Vina RF, Saslavsky J, Urschel HC Jr, Kormos R, et al. Surgical treatment of congestive heart failure with autologous adult stem cell implantation: A prospective randomized study. J Thorac Cardiovasc Surg 2005; 130: 1631-8.
19. Ozbaran M, Omay SB, Nalbantgil S, Kultursay H, Kumanlioglu K, Nart D, et al. Autologous peripheral stem cell transplantation in patients with congestive heart failure due to ischemic heart disease. Eur J Cardiothorac Surg 2004; 25: 342-50 .
20. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 2003; 361: 47-9.
21. Menasché P, Hagège AA, Scorsin M, Pouzet B, Desnos M, Duboc D, et al. Myoblast transplantation for heart failure. Lancet 2001; 357: 279-80.
22. Strauer BE, Brehm M, Zeus T, Köstering M, Hernandez A, Sorg RV, et al. Repair of infracted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002; 106: 1913-8.
23. Stamm C, Kleine HD, Westphal B, Petzsch M, Kittner C, Nienaber CA, et al. CABG and bone marrow stem cell transplantation after myocardial infarction. Thorac Cardiovasc Surg 2004; 52: 152-8.
24. Dimmeler S, Zeiher AM, Schneider MD. Unchain my heart: The scientific foundations of cardiac repair. J Clin Invest 2005; 115: 572-83.
25. Ott H, Davis B, Taylor D. Cell therapy for heart failure - muscle, bone marrow, blood and cardiac derived stem cells. Semin Thorac Cardiovasc Surg 2005; 17: 348-60.
26. Prapas SN, Anagnostopoulos CE, Kotsis VN, Stavropoulos GP, Sidiropoulos AV, Ananiadou OG, et al. A new pattern for using both thoracic arteries to revascularize the entire heart: the π-graft. Ann Thorac Surg 2002; 73: 1990-2.
27. Tector AJ, Kress DC, Downey FX, Schmahl TM. Complete revascularization with internal thoracic artery grafts. Sem Thor Cardiovasc Surg 1996; 8: 29-41.
28. Prapas SN, Protogeros DA, Kotsis VN, Panagiotopoulos IA, Ioannis A, Raptis IP, et al: External reshaping of left ventricle in off pump surgery. Innovations: Technology & Techniques in Cardiothoracic & Vascular Surgery. 2006; 1:155-9.
29. Magginas A, Adamopoulos S, Karavolias G, Kaklamanis L, Saroglou G, Vlahakos D, et al. Orthotopic heart transplantation: Early clinical experience and results of a new transplant center. Hellenic J Cardiol 2003; 44: 102 -7.
30. Angelini P, Markwald RR. Stem cell treatment of the heart. A review of its current status on the brink of clinical experimentation. Tex Heart Inst J 2005; 32: 479-88.
31. Fuchs S, Kornowski R, Weisz G, Satler LF, Smits PC, Okubagzi P, et al. Safety and feasibility of transendocardial autologous bone marrow cell transplantation in patients with advanced heart disease. Am J Cardiol 2006; 97: 823-9.
32. Dor V, Saab M, Coste P, Kornaszewska M, Montiglio F. Left ventricular aneurysm: a new surgical approach. Thorac Cardiovasc Surg 1989; 37:11-9.
33. Dor V, Sabatier M, Di Donato M, Maioli M, Toso A, Montiglio F. Late hemodynamic results after left ventricular patch repair associated with coronary grafting in patients with postinfarction akinetic or dyskinetic aneurysm of the left ventricle. J Thorac Cardiovasc Surg 1995; 110: 1291 -9.
34. Batista RJ, Verde J, Nery P, Bocchino L, Takeshita N, Bhayana JN, et al. Partial left ventriculectomy to treat end-stage heart disease. Ann Thorac Surg 1997; 64: 634-8.
35. Shahzad RG, Benson RS. Levosimendan in cardiac surgery: current best available evidence. Ann Thorac Surg 2006; 81:1536-46 .
36. Ross J Jr. Assessment of ischemic regional myocardial dysfunction and its reversibility. Circulation 1986; 74: 1186-90.
37. Assmann PE, Slager CJ, van der Borden SG, Dreysse ST, Tijssen JG, Sutherland GR, et al. Quantitative echocardiographic analysis of global and regional left ventricular function. A problem revisited. J Am Soc Echocardiogr 1990; 3: 478-87.
38. Lawson MA, Johnson LL, Coghlan L, Alami M, Tauxe EL, Reinert SE, et al. Correlation of thallium uptake with left ventricular wall thickness by cine magnetic resonance imaging in patients with acute healed myocardial infarcts. Am J Cardiol 1997; 80: 434-41.
39. Wu E, Judd RM, Vargas JD, Klocke FJ, Bonow RO, Kim RJ. Visualization of presence of healed Q-wave and non Q-wave myocardial infarction. Lancet 2001; 357: 21-8.
40. Nagel E, Lehmkuhl HB, Bocksch W, Klein C, Vogel U, Frantz E, et al. Noninvasive diagnosis of ischemia induced wall motion abnormalities with the use of high dose dobutamine stress MRI. Comparison with dobutamine stress echocardiography. Circulation 1999; 99: 763-70.
41. Fuster V, Sanz J, Viles-Gonzalez JF, Rajagopalan S. The utility of magnetic resonance imaging in cardiac tissue regeneration trials. Nature 2006; 3 suppl 1: S2-7.
42. Korbling M, Estrov Z. Adult stem cells for tissue repair - a new therapeutic concept? N Engl J Med 2003; 349: 570-82.
43. Assmus B, Schächinger V, Teupe C, Britten M, Lehmann R, Döbert N, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002; 106: 3009-17.
44. Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Hölschermann H, et al.; REPAIR-AMI Investigators. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med 2006; 355: 1210-21.
45. Schächinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI trial. J Am Coll Cardiol 2004; 44: 1690-9.