Autologous peripheral stem cell transplantation in patients with congestive heart failure due to ischemic heart disease

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Abstract

Objective: Ischemic heart disease accounts for 50% of all cardiovascular deaths and is the leading cause of congestive heart failure. Medical therapy, cardiac assist devices and surgical procedures including heart transplantation have limited efficiency and availability. Stem cell transplantation represents a new therapeutic opportunity for such patients. Method: Six patients with the diagnosis of ischemic cardiomyopathy were included in this study. All of the patients had clinical, radiological and echocardiographic signs of heart failure, and reduced left ventricular ejection fraction (LVEF \# 25%). They underwent coronary angiography and stress tests with dobutamine echocardiography, thallium scintigraphy and positron emission tomography to assess myocardial ischemia and viability. Peripheral stem cells were mobilized and collected by apheresis. They were transplanted into areas of injury with open-heart surgery. To increase blood flow to the engrafted areas, coronary artery by-pass surgery was also performed. Results: The patients were followed at least for 4 months. Echocardiography, thallium scintigraphy and positron emission tomography were repeated after at least 6 weeks following surgery. There was a significant increase in life quality and NYHA class. Some benefit was documented on echocardiography, thallium scintigraphy, and positron emission tomography. Conclusion: This approach opens a new window in the treatment of ‘no hope’ patients with congestive heart failure.

Keywords: Stem cell; Congestive heart failure; Transdifferentiation; Cell transplantation

1. Introduction

Ischemic heart disease accounts for 50% of all cardiovascular deaths and is the leading cause of congestive heart failure [1]. The 1-year mortality rate for patients diagnosed with congestive heart failure is 20%. Myocardial infarction is by nature, an irreversible injury [2]. The extent of the infarction depends on the duration and severity of flow cessation. Contraction and fibrosis of myocardium occurs, and regeneration of myocardial tissue cannot always take place after the initial event. To date, no therapeutic procedure like angioplasty and/or thrombolytic agents could reverse the irreversible myocardial injury completely. For this group of patients, which have very low contractile capacity with ejection fractions of lower than 30%, cardiac transplantation and/or mechanic devices stand up as therapeutic choices. However, well compared to the huge number of candidate congestive heart failure patients, the donor supply is strictly limited. Also, mechanical devices are still far from fulfilling the expectations. In the same time, heart transplantation and mechanical device replacements are not suitable for the cost–benefit ratio of the patients. Thus, new therapeutic modalities are enormously needed for the constrained patients.

Recently, regenerative medicine utilizing stem cells from different sources has opened a new window in the therapy of many chronic diseases. The overall objective of stem cell
therapy is to repopulate post-infarction scar tissue with contractile cells that can engraft in sufficient numbers to differentiate to the cardiac myocytes and restore functionality in these akinetic areas [3]. Although cardiomyocytes of infarcted or failing human hearts have been shown to undergo mitosis [4], this regenerative capacity is by far too limited to compensate for the loss of cardiac cells resulting from a large infarct. The biology of stem cells has been enlightened to a great extent by numerous research articles published in the last few years. Among these, especially animal studies related to myocardial regeneration, utilizing different kinds of stem cells have attracted great attention [5–8]. Stem cells from human blastocyst cell cultures, from hematopoietic origin, and from muscle, liver, neuronal tissue have been proved to have the potential for transdifferentiation in in vitro and animal studies. Especially, human blastocyst cells have the greatest potential to differentiate into various tissues. However, ethical considerations limited the usage of these cell lines. Another source of stem cells comparable to blastocyst cells is adult hematopoietic pluripotent stem cells. Hematopoietic stem cells consist of totipotent, mesenchymal and progenitor stem cells. When cultured under appropriate conditions, these cells can differentiate to skeletal and myocardial cells [9]. Also, animal studies with myocardial injury models showed that transplanted hematopoietic stem cells into the injured myocardial tissue could differentiate and regenerate the injured myocardial tissue [10–12]. In the same time, there are reports demonstrating very low numbers of hematopoietic stem cells spontaneously placed in the human myocardial infarct tissue. Stem cell transplantation strategy is likely the most realistic method and consequently has been extensively investigated in the laboratory setting. Most of these experiments have focused on ischemic, segmental cardiomyopathies. Thus, in this highly intriguing study, we intended to evaluate the potential of autologous peripheral stem cells to regenerate the injured myocardial tissue of six congestive heart failure patients due to severe ischemic heart disease.

2. Materials and methods

2.1. Patient selection

The patients with congestive heart failure due to severe ischemic heart disease were taken under evaluation as the target population. The eligibility criteria was as follows; an ejection fraction of lower than 25%, with a poor distal coronary bed not suitable for coronary by-pass surgery, with a range of 35–75 years old, without collagen tissue disease and malignant disease, chronic renal failure, chronic hepatic failure and stroke; without left ventricular aneurysm, without any valvular disease requiring surgical intervention. The accepted patients for the study were the ones evaluated as not suitable for the standard coronary by-pass surgery procedure. These six patients were evaluated clinically and cardiologic/hematologic laboratory analysis including complete blood count and peripheral smear, electrocardiography, echocardiography, dobutamine stress echocardiography, coronary angiography, myocardial TI201 scintigraphy and positron emission tomography (PET).

2.2. Study population

Between June 2002 and December 2002, six patients were enrolled in the study. All of the patients were male with a mean age of 55.5. They all had the diagnosis of ischemic dilated cardiomyopathy with NYHA Class III–IV. Informed consents were obtained and ethical committee approved the patients for the study. All of the patients had clinical, radiological and echocardiographic signs of congestive heart failure and severely reduced left ventricular ejection fraction (LVEF ≤ 50%) assessed by echocardiography (Table 1). The patients underwent coronary angiography and stress tests with dobutamine echocardiography, thallium perfusion scintigraphy (Table 2) and PET (Table 3) to assess myocardial ischemia and viability.

2.3. Coronary angiography

Coronary angiography was performed in multiple projections using Judkins technique. Coronary artery disease was defined as >50% assessed reduction of the lumen diameter of any three coronary artery disease or their primary branches.

2.4. Stress echocardiography

Dobutamine stress echocardiography was performed in all patients. Studies were performed with an ultrasound system (Acuson Cypress Echocardiography System, Siemens Company) using a 3V2c probe. The starting dose was 5 µg/kg per min. The dose was increased at 3-min intervals to 10, 20, 30 and 40 µg/kg per min.

The left ventricle was divided into 16 segments according to the recommendations of American Society of Echocardiography. The wall motion was scored as 1, normal; 2, mildly hypokinetic; 3, severely hypokinetic; 4, akinetic; 5, dyskinetic. A left ventricle wall motion score index (WMSI) was calculated at baseline, low (10 µg/kg per min) and peak dobutamine dosage (Table 1). A segment was defined as viable when during low dose dobutamine systolic wall thickening and endocardial motion appeared basally akinetic or dyskinetic segment; or normal or near-normal wall motion and thickening became apparent in a severely hypokinetic segment. Diagnosis of ischemia was made when (1) a basally akinetic or hypokinetic segment, after improving its thickening and motion at low doses, showed a significant improvement at peak stress (biphasic response); (2) a basally hypokinetic segment showed direct deterioration to akinesia or dyskinesia; and (3) a new asynergia...
developed in a basally normal segment. Akinesia directly deteriorating to dyskinesia was not thought to be indicative of ischemia.

2.5. **Myocardial Ti201 scintigraphy**

Because of the clinical status of the selected patients, myocardial perfusion SPECT was applied only at rest. Rest images were obtained after intravenous injections of 3 mCi Ti201. All data were collected by using Sopha DST dual head gamma camera and low energy-all purpose collimator, and applying 180° SPECT at 64 × 64 matrix. Short axis, horizontal long axis and vertical long axis views were obtained from the data. The medial parts of all short axis, vertical long axis and horizontal long axis views were evaluated. Short axis views were segmented as anterior, septal, lateral and inferior segments; vertical long axis as anterior, apical and inferior, and horizontal long axis as apical, septal and lateral. All the segmental views were scored visually as follows: 0, no activity or activity less than 25%; 1, low activity about 25–50%; 2, less than normal activity about 50–70%; 3, normal activity or activity more than 70% (Table 2).

2.6. **Positron emission tomography**

In order to evaluate the myocardium by PET, all the patients were invited in fasting state. After basal blood glucose count, 50–75 g of glucose was given per oral, and at the end of 30 min, the blood glucose level was aimed to be over 150 mg/dl. The patients were left for a 30-min rest after intravenous injection of 8–10 mgCi F-18-FDG. At the end of this procedure, visualization was obtained by zooming the cardiac region by PET camera. Visualization procedures were completed by Siemens Ecat EXACT PET camera. After locating the cardiac regions by ‘Exact ACQ scout EM’ visualization protocol, emission views were taken for 20 min, and then transmission views for 5 min by ‘Exact ACQ Heart 3D EM + Tx(hot)’ protocol. Backprojection filters were used for analysis. By the help of computer systems, short axis, horizontal long axis and vertical long axis views were obtained. Short axis views were segmented as anterior, septal, lateral and inferior segments; vertical long axis as anterior, apical and inferior; and horizontal long axis as apical, septal and lateral. All the segmental views were scored visually as follows: 0, no activity or activity less than 25%; 1, low activity about 25–50%; 2, less than normal activity about 50–70%; 3, normal activity or activity more than 70% (Table 3).

### Table 1
The echocardiographic variables of the patients

<table>
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<tr>
<th>TTE</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
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<tr>
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<td>57</td>
<td>69</td>
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<tr>
<td>Lves (mm)</td>
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<td>42</td>
<td>53</td>
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<td></td>
<td>After</td>
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<tr>
<td>Infarct territory</td>
<td>LAD, RCA</td>
<td>LAD, RCA</td>
<td>LAD, RCA</td>
<td>LAD, RCA</td>
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<tr>
<td>WMSI</td>
<td>2.81*/2.3**</td>
<td>2.6*/2.3**</td>
<td>2.87*/2.25**</td>
<td>3.0*/2.8**</td>
<td>2.3*/2.1**</td>
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</tr>
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</table>

TTE, transthoracic echocardiography; Lved, left ventricle end-diastolic diameter; Lves, left ventricle end-systolic diameter; IVS, interventricular septum; PW, posterior wall; LVEF, left ventricular ejection fraction; RVEF, right ventricular ejection fraction; DSE, dobutamine stress echocardiography; WMSI, wall motion score index; *, WMSI at rest; **, WMSI at low-dose DSE.

2.7. **Mobilization and collection of stem cells**

After each patient was evaluated by complete blood count and peripheral smear, they were accepted as hematologically normal. The mean peripheral leucocyte count was between 5000 and 10 000 mm⁻³, hematocrit between 38 and 42% and platelet counts between 200 000 and 450 000 mm⁻³. No atypical cell or reactive lymphocytosis was observed. The absolute counts of the differential were in normal range. After obtaining informed consent, G-CSF (Neupogen®, Roche Pharmaceuticals) 30 × 10⁶ i.u./day was administered subcutaneously to each patient. Blood counts, peripheral smears and peripheral CD34 + cell counts were determined daily. After approximately 4 days of G-CSF administration, sufficient number of stem cells were mobilized to peripheral blood. Peripheral blood stem cell collection was applied by Fresenius AS 204 or Amicus, Baxter apheresis systems by appropriate softwares. A mean 60–80 cm³ of mononuclear cell collection was obtained in suspension form. The CD 34 + absolute
Table 2
Thallium myocardial scintigraphy showing pre- and post-transplantation

<table>
<thead>
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<th>Patient</th>
<th>Short axis</th>
<th>Horizontal long axis</th>
<th>Vertical long axis</th>
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<td>1</td>
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<td>3</td>
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<td>6</td>
<td>3</td>
<td>3</td>
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</table>

0, 1, 2 and 3, the scores are described in Section 2; *, not determined.

Table 3
PET showing pre- and post-transplantation

<table>
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<th>Horizontal long axis</th>
<th>Vertical long axis</th>
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<td>Septum</td>
<td>Lateral</td>
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<td>1</td>
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<td>4</td>
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0, 1, 2 and 3, the scores are described in Section 2; *, not determined.
stem cell numbers ranged between $13 \times 10^6$ and $80 \times 10^6$ cells. Absolute mononuclear cell counts ranged between $4.5 \times 10^9$ and $63.5 \times 10^9$ cells. The cell suspensions were not cryopreserved, and were kept at 4 °C overnight by gentle shaking. Before transplantation of the cells, cell viability was determined by trypan-blue-dye exclusion test and it was found to be over 95% viable in each sample.

2.8. Transplantation of stem cells

The standard anesthetic procedures appropriate for low-EF patients were applied. An intravenous phentanyl-based anesthetic technique was administered. Under general anesthesia, all the patients were prepared by median sternotomy, and standard cardiopulmonary by-pass was established. The standard coronary by-pass procedure was performed in all. We intended to establish the blood flow to the transplantation areas by this procedure. During arrest time, the stem cell suspension was injected either to the injured non-fibrotic myocardial tissue surrounding the anastomosis site by small increments radially or via the coronary arteries, which were opened for coronary anastomosis. With the aim of achieving graft-contact and complete engraftment, the transplantation was applied to myocardium circumferentially. Afterwards, the operation was terminated with standard techniques. Autologous or allogeneic erithrocyte suspensions were infused as required.

2.9. Follow up

The patients were followed for 4–10 months. Two-dimensional echocardiography was applied at day 7 of operation and every month after discharge. TI$^{201}$ perfusion scintigraphy and PET imaging were repeated after at least 6 weeks following operation.

3. Results

3.1. Patient 1

S.E., male, 65 years old. Myocardial injury had occurred 4 months before stem cell transplantation. He had no chest pain; he was in NYHA Class III before the procedure. LVEF was 25%. He had two-vessel disease. PET, TI$^{201}$ scintigraphy and dobutamine stress test were negative for ischemia. He had infarction within LAD and RCA territories with no viability. He had LIMA and saphenous vein grafts for LAD and RCA, respectively. Meanwhile, a total number of $19 \times 10^6$ stem cells were transplanted. His post-operative period was uneventful, and he was discharged from the hospital on seventh day. He is followed for 10 months. Clinically, there is a significant improvement to NYHA Class II. Echocardiography showed improvement (Table 1). TI$^{201}$ scintigraphy (Table 2) and PET (Table 3) demonstrated significant increase with clear viability in the LAD territory without any viability preoperatively.

3.2. Patient 2

M.D., male, 52 years old. He had myocardial infarction 2 years before stem cell transplantation. Although he had no chest pain, he had dyspnea on exertion. He was in NYHA Class III before the procedure. LVEF was 20%. He had multivessel disease with poor distal vasculature. No ischemia was detected on PET, TI$^{201}$ scintigraphy and dobutamine stress tests. He had infarction within LAD and RCA territories with minimal viability limited to the apical segment. He had LIMA graft for LAD. A total number of $21 \times 10^6$ stem cells were implanted. His post-operative period was uneventful, and he was discharged on seventh day. He is followed for 10 months. He is now in NYHA Class II. There is no significant improvement on echocardiography (Table 1), TI$^{201}$ scintigraphy (Table 2) and PET (Table 3) post-operatively.

3.3. Patient 3

A.I., male, 58 years old. He had myocardial injury 4 years before the procedure. He was in NYHA Class III before the procedure. LVEF was 24%. He had three-vessel disease. PET, TI$^{201}$ scintigraphy and dobutamine stress tests were negative for ischemia. He had infarction within LAD and RCA territories with viability limited to the basal anterior segment. He had LIMA and saphenous vein grafts for LAD, OM$_1$ and RCA, respectively. A total number of $80 \times 10^6$ stem cells were transplanted. In his post-operative period, on the second day, he had high ventricular rate atrial fibrillation. He was resistant to drug therapy, and he maintained sinus rythm after electrical cardioversion. He was discharged from the hospital on 13th day. After 6 weeks, he was rehospitalized in sepsis. His LVEF was 20% on echocardiography (Table 1), but the other tests could not be repeated. He died within 24 h of hospitalization. Necropsy of the myocardium was performed with the consent of the family.

3.4. Patient 4

C.G., male, 69 years old. He had myocardial injury 10 years before the procedure. A year before stem cell implantation, he had progressively increasing dyspnea on effort. He was in NYHA Class III. LVEF was 20%. He had three-vessel disease. PET, TI$^{201}$ scintigraphy and dobutamine stress tests showed no ischemia. He had infarction within LAD and RCA territories without any viability. He had LIMA and saphenous vein grafts for LAD, OM$_1$ and OM$_2$, respectively. A total number of $46.5 \times 10^6$ stem cells were transplanted. Mediastinitis due to coagulase-(−) S. aureus developed in his post-operative period, and he was discharged from the hospital on 54th day. He is
followed for 9 months. He was rehospitalized with the diagnosis of congestive heart failure on 4th month. He did well after his discharge. In his follow up, he improved considerably from the clinical point of view by changing to NYHA Class II. On his control echocardiography, his LVEF improved 25% compared to preoperative values (Table 1). However, no changes were detected on Tl201 scintigraphy (Table 2) and PET (Table 3).

3.5. Patient 5

B.E., male, 41 years old. Myocardial infarction had occurred 1 month before stem cell transplantation. He had no chest pain; he was in NYHA Class IV before the procedure. LVEF was 17%. He had two-vessel disease. PET, Tl201 scintigraphy and dobutamine stress test were negative for ischemia. He had infarction within LAD and RCA territories with viability limited to the septum. He had LIMA and saphenous vein grafts for LAD and RM, respectively. A total number of 13.5 × 10⁶ stem cells were transplanted. His post-operative period was uneventful, and he was discharged from the hospital on ninth day. He is followed for 9 months. He was rehospitalized on fifth month because of pneumonia. No improvement was detected on echocardiography (Table 1) and Tl201 scintigraphy (Table 2), but an increase in viability in apex was detected by PET (Table 3).

3.6. Patient 6

S.Y., male, 48 years old. He had myocardial infarction within 3 months before stem cell transplantation. He had no chest pain; he was in NYHA Class III before the procedure. LVEF was 20%. He had two-vessel disease. PET, Tl201 scintigraphy and dobutamine stress tests were negative for ischemia. He had infarction within LAD territory without any viability. He had LIMA and saphenous vein grafts for LAD, OD and RCA, respectively. A total number of 15.7 × 10⁶ stem cells were transplanted. His post-operative period was uneventful, and he was discharged from the hospital on 13th day. He is followed for 4 months. There is marked improvement in his clinical status. He is now in NYHA Class I. On echocardiography, LVEF is 45%, and LV dimensions are in normal ranges (Table 1). Tl201 scintigraphy (Table 2) and PET (Table 3) demonstrated marked improvement in anterior, septal and apical regions.

4. Discussion

Despite an overall decline in cardiovascular disease mortality in industrialized countries over the last two decades, chronic heart failure continues to be a major health care problem [1]. Surveys indicate that heart failure affects 1–2% of the population in western countries. The prevalence of heart failure has been shown to rise sharply and may increase by as much as 70% by the year 2010. It has significant mortality and morbidity. Prognosis is extremely poor once the symptoms and clinical signs of heart failure become overt, and the majority of patients die within 5 years after diagnosis. The main reason of mortality is the lack of efficient therapy in NYHA Class III and IV patients. Heart transplantation has been the most effective treatment for this group of patients. However, it has a number of drawbacks, like coronary vasculopathy, rejections, the need for life-long immunosuppression and scarcity of heart donors. Dynamic cardiomyoplasty has not been quite successful. Artificial heart devices also are still far from perfection with complications such as infection, thrombosis, hemolysis and high cost. Xenogenic heart graft researches are continuing, but are still far from clinical utilization. Thus, new therapeutic approaches are desperately needed for this end-stage, ‘no hope’ patients. Stem cell transplantation represents an opportunity for developing new therapeutic strategies for such patients.

It is accepted that adult cardiomyocytes lack the ability to regenerate the myocardium. Cellular research on animal models demonstrated that cardiomyocyte regeneration could be possible in the infarct areas. Different sources of cells are investigated for this purpose. The most primitive of all stem cell population are the embryonic stem cells. These are the inner cell mass at day 5 after fertilization that have vast potential for development. It is well shown that these cells can differentiate to cardiomyocytes in both animal and human studies. However, allogeneic origin of these cells causes immunological problems and lifelong immunosuppressive drugs are needed to prevent rejection. More importantly, use of these cells in research causes moral and ethical problems.

Satellite muscle cells or myoblasts are the precursor cells of skeletal muscle that have capacity for self-renewal and differentiation. Myoblast transplantation have been performed in animal models [13]. Although cardiomyogenic transdifferentiation of myoblasts and improvement of ventricular function have been shown, the new cells lack the characteristics of real cardiac cells like cardiac specific proteins, gap junctions, and electrophysiological properties. Skeletal muscle transplantation is already being applied clinically. Menasché et al. [14] reported successful implantation of autologous skeletal myoblasts in 10 patients with ischemic cardiomyopathy. The functional status of the patients improved after the transplantation. The ejection fraction on echocardiography was improved from 25 to 35%. But ventricular arrhythmia necessitating amiodarone and Internal Cardioverter Defibrillator (ICD) therapy complicated 7 of the 9 surviving patients. In another study from USA [15], nine patients undergoing CABG and four patients undergoing left ventricular assist device implantation were transplanted with myoblasts. At the end of a 6-month follow up period, viability in the grafted scar was shown by MRI and PET scanning. Although the authors concluded that myoblast transplantation is safe and feasible,
long-term effects of skeletal myoblasts, especially their probable arrhythmogenic substrate need to be confirmed.

Adult hematopoietic stem cells (AHSC) showed to have near potential to blastocyst cells to transdifferentiate crossing embryonal layers. Under appropriate culture conditions with suitable medium and growth factors, they can transdifferentiate to cardiomyocytes, skeletal muscle cells [16], vascular endothelium, endodermal cells, hepatocytes and neural cells. Animal infarct models were used to test cardiomyocyte regeneration with AHSC. Significant increases in myocardial contractility and clonal cardiomyocyte regeneration were demonstrated by molecular biology techniques. Transplanted cells behave as platforms releasing growth and/or angiogenic factors [17]. Preclinical models have shown the ability of undifferentiated human mesenchymal cells to undergo site specific differentiation into a functional cardiac muscle phenotype after injection into sheep [18]. Another subset of bone marrow stromal cells referred to as mesodermal progenitor cells or multipotent adult progenitor cells has been described. Multipotent adult progenitor cells copurify with mesenchymal stem cells. They proliferate extensively and differentiate in vitro into cells of all three germ layers. When injected in vivo, they reconstitute bone marrow, liver, gut, lungs, and endothelium. In the treatment of blood disorders, it is now routine clinical practice to isolate and transplant CD34 + stem cells. These include progenitor cells and stem cells that provide short- and long-term hematopoietic reconstitution. Bone marrow stem cells have the ability to differentiate into stem and progenitor cells that mature into functional cells in a variety of tissues including myocardium [19]. The cytokine G-CSF is widely used to mobilize stem/progenitor cells that are harvested by leukapheresis, stored, and subsequently reinfused to support hematopoietic recovery in patients after chemotherapy or radiation treatment.

Orlic et al. [20] demonstrated regeneration in the mouse myocardial infarction model with G-CSF mobilized autologous bone marrow stem cells. Strauer et al. [21] reported autologous mononuclear bone marrow transplantation via the infract related artery after angioplasty in 10 patients with improvement. The hematopoietic subpopulations of stem cells responsible from differentiation/transdifferentiation are not clear yet, especially in in vivo conditions. Clinical experience shows that bone marrow stem cell transplantation does not lead to neoplasia so far. Although the mesenchymal stem cells represent an ideal cell population for organ regeneration, the use of the entire mobilized mononuclear cell fraction should be favored, since hematopoietic stem cells, their progenitor cells and endothelial progenitor cells are present in addition to mesenchymal stem cells. Hamano et al. [22] showed in five patients that autologous bone marrow cells can be injected safely during a by-pass operation into areas of ischemic myocardium that could not be treated with a by-pass graft. Brehm et al. [23] have treated 23 patients with acute cardiac infarction using autologous mononuclear bone marrow cells.

In this study, we intended to give a stem cell support to the no-hope group of patients. From hematological peripheral blood stem cell research, it is well known that, after induction of stem cell pool in bone marrow by appropriate growth factors, different kinds of AHSC can be enriched in the peripheral blood including mesenchymal, dendritic, progenitor and pluripotent stem cells. We utilized G-CSF for the purpose of mobilization. By apheresis technology, these cells are collected and concentrated in the mononuclear cell fraction of peripheral blood. We processed approximately 8–15 litre of blood to reach sufficient stem cell numbers. We did not perform further purification or isolation of different kinds of stem cells with the data in mind that the accessory cells in the mononuclear cell compartment are also essential for engraftment and transdifferentiation. The animal data of Orlic et al. showed that after GCSF mobilization, there was not any implantation and new tissue formation by mobilized stem cells. Therefore, we evaluated the new vessel formation in the necropsy material as a consequence of transplanted stem cells. This is a novel technique to harvest stem cells that has not been applied in animal or human studies previously. We transplanted stem cells by injection to the border of infarct area and by infusion through the bypass grafts to assure adequate delivery of these cells and to enrich the blood supply to these areas. All of the patients were weaned off from cardiopulmonary by-pass without need of high dose inotropic agents or IABP. Post-operative period was uneventful. Atrial fibrillation developed in one patient. Sinus rhythm was restored within 48 h with amiodarone therapy and electrical cardioversion. Ventricular arrhythmia was not observed.

One patient was hospitalized in septic shock and died. No source of infection could be demonstrated. Autopsy was performed. On pathological examination of the heart, tissue sections were stained for factor VIII, CD34 and CD31 to detect endothelial cells (Fig. 1A–C). The number of capillaries was increased and new capillary formation was detected within the area of infarction. However, myoblast or fibroblast activation could not be demonstrated. We believe that the formation of new capillaries can explain the improved perfusion detected on PET.

All five patients showed clinical improvement. Although all of them improved from NYHA Class III–IV to Class I–II, ejection fraction on echocardiography got better in only three of the patients. Two of these patients had significant viability in the infarct areas on scintigraphy and PET when compared to preoperative values (Figs. 2 and 3, respectively). The improvement of ejection fraction started to become evident on echocardiographic examination performed on the second month. One interesting observation was that the two patients who benefited most were the ones with recent myocardial infarction.

One may argue that the beneficial effects of CABG may have influenced the improvement of the patient’s clinical situation. We believe that overall improvement cannot solely be due to the revascularization since no ischemia nor
viability were detected on dobutamine echocardiography and PET preoperatively.

Our study is one of the first clinical series demonstrating benefit of autologous adult hematopoietic stem cell transplantation. There are two other studies from the UK and Germany showing some benefit in their patients. Galinanes et al. from UK [24] transplanted bone marrow cells to 14 patients undergoing CABG. The patients were evaluated only by echocardiography in their study. Some improvement of wall motion was detected on dobutamine echocardiography. In another study from Germany, eight patients underwent cell transplantation and CABG [25]. Improvement of myocardial perfusion was detected on thallium scintigraphy after a median follow up of 10 months. No evidence of adverse effects like ventricular arrhythmia or neoplasm were detected in these studies.

We believe that this is an impressive and new therapeutic window for the no-hope heart failure patients. It is feasible and safe. No adverse effect was detected in the short term. We speculate that patients with recent myocardial infarction may benefit more from cell transplantation. This is a preliminary study with limited number of patients. Further research is needed to confirm these results.

Fig. 1. The pathologic specimens of ‘Patient 2’ with the necropsy performed 6 weeks after transplantation. The arrow-heads point to the angiogenic buds around the needle puncture. (A) Hemotoxylin–Eosin 10 x, (B) CD34 staining, (C) CD31 staining.

Fig. 2. The thallium scintigraphic example for ‘Patient 1’. (A) The preoperative study. (B) The post-transplant study after 7 months. S.A., short axis; H.L.A., horizontal long axis; V.L.A., vertical long axis; ant, anterior; sep, septum; inf, inferior.

Fig. 3. The positron emission tomographic example for ‘Patient 6’. (A) The preoperative study. (B) The post-transplant study after 4 months. S.A., short axis; H.L.A., horizontal long axis; V.L.A., vertical long axis; ant, anterior; sep, septum; inf, inferior.
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References


Appendix A. Editorial comment

**Cellular cardiomyoplasty: ‘infect easy, demonstrate is complicated’**

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In the last decade, improvements in cell biology, cell selection and cell culture have led to the development and refinement of a new strategy to treat ischemic and post-infarction heart diseases [1]. The goal for cell therapy or cellular cardiomyoplasty is to repopulate the scar, to develop new contractile activity, and eventually to improve scar perfusion. Cell therapy offers cardiac surgeons new fields of experimental and clinical research for treating infarcted and ischemic cardiac tissues [2]. Two of the most widely used cell types in this new strategy are skeletal muscle-derived progenitors or myoblasts and mononuclear bone marrow-derived cells (BM-MDC). These cells are autologous and easily expandable in vitro. The relative limitation of myoblasts is maintained in muscle phenotype [2]. However, they are well integrated in the scar and are able to improve cardiac function. In contrast, BM-MDC are potentially interesting for their capacity to transdifferentiate into cardiomyocytes and endothelial cells. BM-MDC are...