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Original Article

Encouraging Experience with Intracardiac Transplantation of Unselected Autologous Bone Marrow Cells Concomitant with Coronary Artery Bypass Surgery after Myocardial Infarction

Sebastian Holinski, MD,[1] Birte Schmeck, MD,[1] Benjamin Claus, MD,[1] Hartmut Radtke, MD, PhD,[2] Thomas Elgeti, MD,[3] Martin Holzhausen, PhD,[4] and Wolfgang Konertz, MD, PhD[1]

Background: Chronic heart failure after myocardial infarction is still a serious problem without a fundamental therapy. Experimental transplantation of bone marrow cells (BMC) into infarcted myocardium resulted in regeneration and functional improvement.
Objective: Clinical investigation of safety and efficacy of intracardiac transplantation of unselected autologous BMC. Method: 22 patients scheduled for elective and isolated coronary artery bypass grafting (CABG) with a reduced LVEF due to myocardial infarction were included. Intraoperatively, sternal bone marrow blood was aspirated, and a sterile buffy coat was prepared and applicated. 19 age, LVEF and coronary disease matched patients served as controls. Heart function, geometry, and scar proportion were assessed by echocardiography and Gadolinium-MRI at the time of the operation and 6 months thereafter.
Results: Transplanted patients received a mean number of 360×10^6 BMC. We did not notice any significant differences in early or late complications in the transplant group as compared to controls. At six months follow up only the transplanted patients showed a significant improvement of NYHA classes from 2.7 to 1.5 and of LVEF from 36 to 43 %, ($p < 0.05$). Furthermore, only CABG concomitant with BMC-TX led to a significant reduction of left ventricular end diastolic diameter (LVEDD) from 59 to 54 mm and of scar proportion of the infarcted segments from 2.53 to 2.42, ($p < 0.05$).
Conclusion: Intracardiac transplantation of unselected, autologous BMC is safe and feasible. In adjunct with coronary revascularization it leads to an improvement of ventricular geometry and function. Moreover, it reduces myocardial scar proportion and heart failure symptoms.

[1]*Department of Cardiovascular Surgery, Charite, Humboldt University, Berlin, Germany*
[2]*Institute of Transfusion Medicine, Charite, Humboldt University, Berlin, Germany*
[3]*Department of Radiology, Charite, Humboldt University, Berlin, Germany*
[4]*Department of Biometry, Charite, Humboldt University, Berlin, Germany*

Received: February 16, 2010; Accepted: October 13, 2010
Corresponding author: Sebastian Holinski, MD. Departments of Cardiovascular Surgery, Humboldt University, Berlin, Germany
Email: sebastian.holinski@charite.de

Introduction

Referring to an estimation of the European society of cardiology, there is a prevalence of symptomatic chronic heart failure (CHF) of 0.4 to 2 % in the European population, resulting in at least 10 million people suffering.[1)] Myocardial infarction is the most common etiology of CHF. Prognosis of this chronic disease is uniformly poor because no established therapy is available to reconstitute lost myocardium.

However, due to exciting, experimental research, the concept of intracardiac application of autologous bone

marrow cells was developed. Makino et al showed that adult bone marrow stem cells are able to differentiate into cardiomyocytes.[2] Thereafter, experimental intracardiac transplantation of bone marrow cells after myocardial infarction was performed. It led to an improvement in heart function and infarct size reduction by the newly formed myocardium.[3, 4]

Based on promising experimental results, bone marrow cell transplantation was introduced clinically to treat ischemic cardiomyopathy. It was shown that bone marrow cells can be applicated safely catheter-based, as well as surgically.[5, 6] Different, complex preselection and cultivation procedures were commonly performed before transplantation. Functional, geometric, as well as morphologic parameters of diseased hearts were more or less positively influenced, depending on the selected cell types and application procedures. In order to simplify the cell harvest and to evaluate the complex transplant effect of pure bone marrow cells we transplanted unselected, noncultivated bone marrow cells. Herein, we report our experience concerning safety and mid term efficacy of intracardiac transplantation of this strategy in patients with ischemic cardiomyopathy concomitant with coronary artery bypass grafting (CABG).

Method

Study design

A prospective pilot study was conducted. Patients scheduled for elective and isolated, primary CABG with a reduced LVEF (< 50%) due to myocardial infarction (Q-wave in ECG and corresponding occluded coronary vessel in angiography) were included. Ischemic cardiomyopathy was evaluated concerning global and regional myocardial function using trans-thoracic echocardiography, as well as cardiac catheterization. Clinically unstable patients and those with a known hematologic disorder were excluded. A control group matched for age, coronary artery disease (CAD), and LVEF was created. The Study was approved by the local ethical board, and informed consent of patients was obtained.

Bone marrow cell harvest, preparation and transplantation

Bone marrow cell harvest and transplantation was performed during the coronary artery bypass operation. 100 ml heparinized sternal bone marrow blood was aspirated before median sternotomy. A sterile buffy coat was prepared after 12 min of centrifugation (4000 G).

Coronary artery bypass grafting using normothermic cardiopulmonary bypass and warm blood cardioplegia was performed during bone marrow cell preparation. Cell transplantation was conducted during cardioplegic arrest after the distal anstomoses were completed. If the infarcted area was clearly visible, the cell suspension was directly injected into the scar. If the infarcted area was not visible, but an infarct coronary vessel was bypassed, cell application was done via a bypass graft to the infarct region. If the infarction area was neither visible nor adequate for bypass surgery, bone marrow cells were infused into the aortic root. Afterwards, proximal anastomoses were performed, and the patient was weaned from cardiopulmonary bypass. The chest was closed in a standard fashion.

Cardiac assessment and follow up

In order to assess functional, as well as morphologic cardiac changes, trans-thoracic echocardiography (HP Sonos 5500, Hewlett-Packard Company, Palo Alto, USA) and Ga-MRI (Siemens Magnetom Sonata, 1,5 T, Siemens, Erlangen, Germany) were performed at the time of the operation and six months after the operation. Left ventricular ejection fraction and left ventricular diameter were measured. Scar proportions were evaluated in a standardized 17-segment model.[7] They were estimated and scored as follows (0 = 0%, 1 = 1%–25%, 2 = 26%–50%, 3 = 51%–75%, 4 = 76%–100%). At follow up medical history was obtained and physical examination performed. Cardiac symptoms, events and interventions, as well as neoplastic diseases, were of special interest.

Statistics

Statistical analysis was done using SPSS® 14.01 (SPSS Inc., Chicago, USA). The level of significance was $\alpha = 0.05$.

Results

Operative and Early postoperative Results

41 patients were included into the study, see **Table 1** for patients characteristics. 22 patients received a mean number of 360×110^6 (median 388×110^6, range 18.7–1596×10^6) autologous bone marrow stem cells concomitant with an average of 3 coronary bypasses. There were 16 applications via bypass grafts, four via the aortic root and two intramuscular injections of cells. No intraoperative complications occurred in either group. Two patients of the transplant group received an intra-aortic balloon pump (IABP) intraoperatively which were weaned

Table 1 Preoperative patient characteristics

	Tx-Group Number			Controls Number			p
Patients	22			19			
Male gender ♂	18			15			ns
	Mean	Median	Range	Mean	Median	Range	
Age, yrs	68	72	44–84	69	69	47–84	ns
CAD	3	3	2–3	3	3	2–3	ns
LVEF, %	36	35	15–50	37	40	25–45	ns
Log. Euroscore, %	6	6	1–14	7	5	1–20	ns

CAD, coronary artery disease; LVEF, left ventricular ejection fraction; n, non-significant

Table 2 Operative and ICU data

	Tx-Group Mean	Median	Range	Controls Mean	median	Range	p
Bypasses, #	3	3	1–6	2.8	3	1–4	ns
Aortic crossclamp t, min	46.0	43.0	18–88	40.4	39.0	20–72	ns
Cardiopulmonary bypass t, min	75.0	76.5	29–120	63.7	60.0	37–118	ns
Ventilation t, h	14.3	9.5	2–70	12.4	12.5	6–22	ns
Inotropic support t, h	15	8.5	0–72	12.4	6	0–63	ns

without problems at the 2nd and 3rd postoperative day. For more periperative data see **Table 2**. We did not notice any significant differences in early postoperative complications in the transplant group as compared to controls. There were no tamponades, re-explorations for bleeding, gastrointestinal complications, mediastinitis, renal failures or cerebral vascular accidents. However, one patient of the transplant group died at the 18th post-operative day due to myocardial infarction, resulting in acute cardiogenic shock. Pathological examination showed thrombosis of two venous bypasses in the presence of a heparin induced thrombocytopenia and very tiny target vessels.

Follow-up results

Six months after the operation one patient had died in each group, both for noncardiac reasons. PCI had been performed in one transplanted patient, but no patient underwent coronary reoperation. An automatic implantable cardioverter defibrillatot (AICD) was implanted in three patients who have had cell transplantion, as well as in two patients of the control group. Apart from one control patient who suffered from liver cancer, no neoplasms were noticed.

NYHA classes at follow up had improved in both groups. NYHA of transplanted patients increased significantly from 2.7 to 1.5 ($p < 0.05$), whereas control patients showed a smaller, non-significant improvement from 2.4 to 1.9 ($p = 0.3$).

Echocardiographic/MRI results

At six months follow up, the transplanted patients showed a significant increase of their LVEF from 36% (median 40, range 15–50, SD ± 9.7) to 43% (median 45, range 30–55, SD ± 7.4) ($p = 0.048$). LVEF of the control patients increased from 37% (median 40, range 25–45, SD ± 7.3) to 41 % (median 42, range 30–55, SD ± 8.1) six months after the operation, ($p = 0.14$), see **Figure 1**. Patients who received the cells via the aortic root had the greatest LVEF increase. Their LVEF rose from 26 % before the operation to 40% at follow up. Intramyocardial transplantation led to no increase at all. Patients transplanted via the bypass graft improved from 36% to 43% resembling, the overall results.

A significant reduction of LVEDD of all transplanted patients from 59 mm (median 59, range 44–80, SD ± 9.8) to 54 mm (median 56, range 40–64, SD ± 7.4) was seen at follow up, $p = 0.025$. LVEDD of controls decreased non-significantly from 56 mm (median 57, range 44–67, SD ± 7.1) to 55 mm (median 56, range 44–68, SD ± 7.4), $p = 0.449$. LVEDD decreased most after intra-aortic transplantation of bone marrow cells (63 vs 53 mm). Intramyocardial injection and injection via bypass grafts led to a reduction of LVEDD from 51 to 47 mm and 58 to 53 mm, respectively. Please see

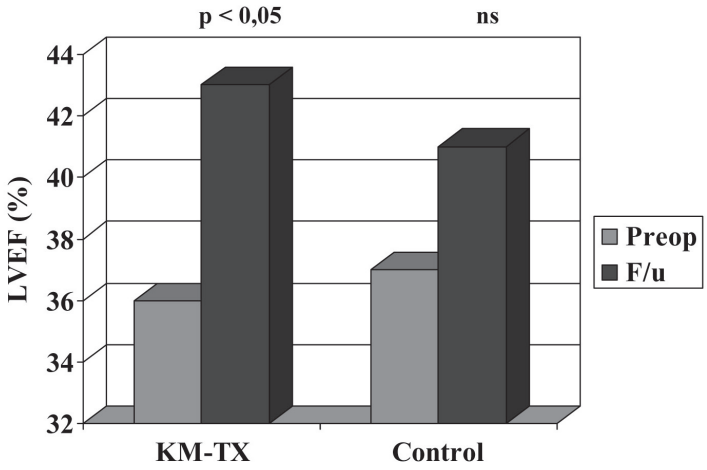

Fig. 1 Mean LVEF of BMC-transplanted patients versus controls, before and 6 months after the operation (F/u).

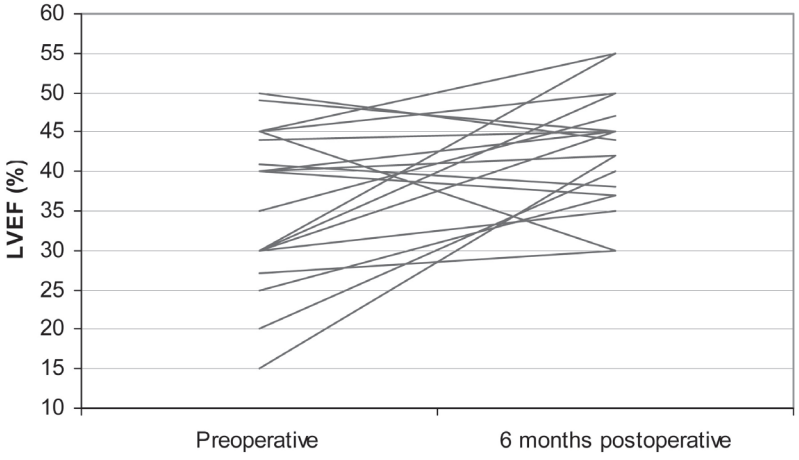

Fig. 2 Individual LVEF course of BMC-transplanted patients, before and 6 months after the operation.

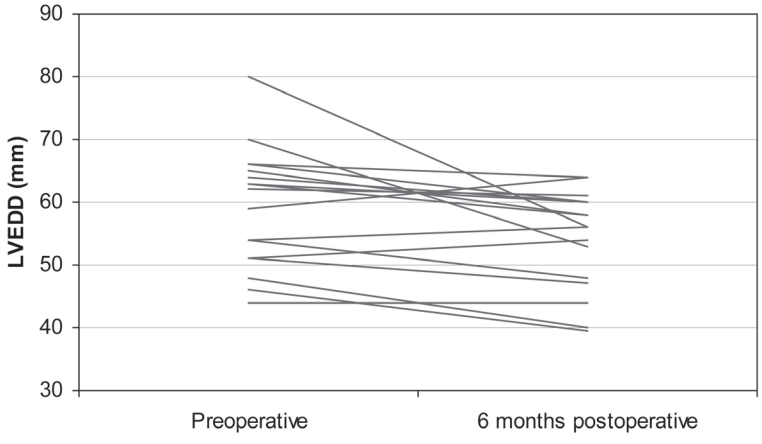

Fig. 3 Individual LVEDD course of BMC-transplanted patients, before and 6 months after the operation.

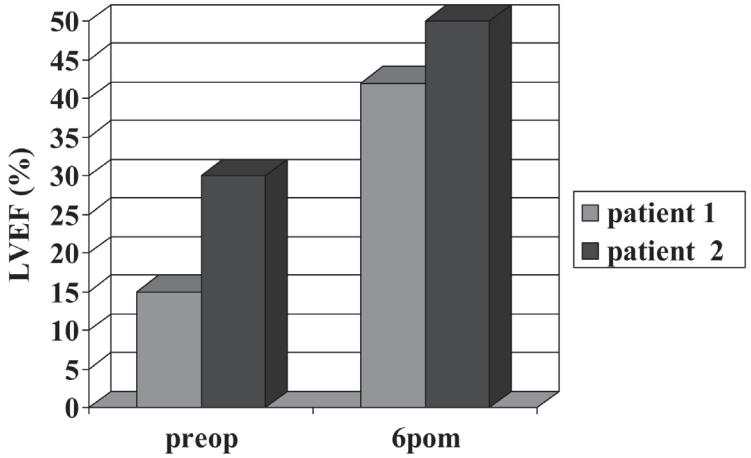

Fig. 4 Examples of postoperative course of LVEF after BMC-Tx (patients of **Table 3**).

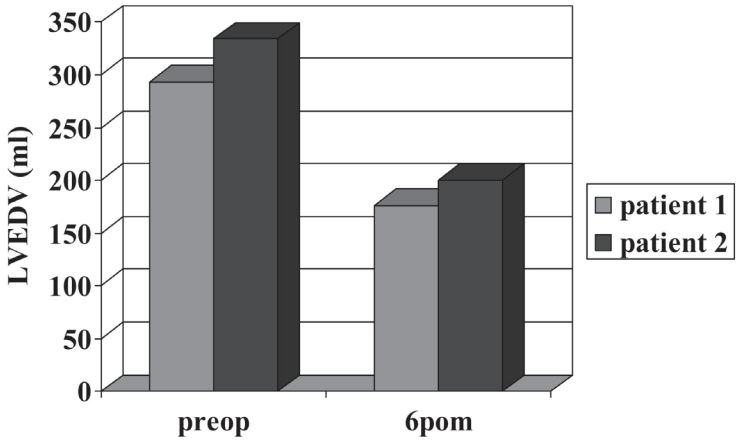

Fig. 5 Examples of postoperative course of LVEDV after BMC-Tx (patients of **Table 3**).

Figure 2 and **Figure 3** for individual LVEF and LVEDD courses of BMC-transplanted patients, as well as **Figure 4** and **Figure 5,** for patient examples related to **Table 3**.

Mean scar score of all segments did not show a significant change, neither in the tx-group (1.14 vs 1.11, ns) nor in the control group (0.63 vs 0.66, $p = 1.0$). There was also no relevant change of the infarct segments of the control patients (1.78 vs 1.81, $p = 1.0$). However, there was a relevant change of the scar proportions of the infarcted segments in the transplanted patients seen at follow up (2.53 vs 2.42, $p < 0.05$).

Discussion

Cell transplantation to treat ischemic heart failure is based on the concept to replace lost contractile units. Initially, only cells from contractile tissues like skeletal myoblasts were used, but they lack complete myocardial integration and failed to improve heart function after myocardial infarction[8] However, experimental results showed the ability of cells from the bone marrow to differentiate into cardiomyocytes.[2] Therefore attempts focused on this primarily noncontractile cell source. Encouraging experimental results after cell transplantation of bone marrow stem cells into infarcted hearts triggered clinical introduction of intracardiac BMC transplantation.[3]

Interventional attempts focused on treatment of patients early after acute myocardial infarction whereas surgery concentrated on patients suffering from chronic ischemic heart failure.[5, 6] Different bone marrow cells

Table 3 Examples of postoperative course of LVEF and LVEDD/LVEDV after BMC-Tx

Patients	#1 (72 y/o male)	#2 (59 y/o male)
CABG procedure	3-CABG (LIMA-LAD, seq. ACVB to Dg-OM)	5-CABG (LIMA-LAD, seq. ACVB to OM1-OM1-RCX, ACVB to RCA
Way of BMC-TX	Intra-aortic	via sequential ACVB
Preop LVEF (TTE)	15 %	30
6pom LVEF (TTE)	42 %	50
Preop LVEF (MRI)	24 %	22
6pom LVEF (MRI)	41 %	54
Preop LVEDD (TTE)	64	66
6pom LVEDD (TTE)	-	60
Preop LVEDV (MRI)	293 ml	334 ml
6pom LVEDV (MRI)	176 ml	200 ml

Preop, preoperative; 6pom, six months after the operation; TTE, trans-thoracic echocardiography; MRI, magnetic resonance imaging

types have been used commonly after selection and cultivation.[9, 10] CD133 + cells were probably most frequently used and proofed to be efficient. However, preparation of these cells is very time and cost intensive. Special equipment is needed, which is not routinely available. Unfortunately this led to the de-randomization of the Rostock-trial.[9] Furthermore preselection of bone marrow cells might exclude other potentially effective cells. Therefore, we decided to transplant unselected and uncultivated bone marrow cells, which allows to harvest and transplant the cells during the same procedure.

Our results demonstrated safety and efficacy of this strategy. No adverse events associated with cellular transplantation were noticed. Indication for all AICD implantations was the MADIT II study based on a LVEF < 35%.

Cardiac geometry, function, scar proportion and NYHA-class improved significantly six months after the operation, only in patients who received autologous BMC in adjunct with CABG. Therefore these effects can be attributed to cell transplantation and not to bypass revascularization alone. Furthermore, we did not see a correlation between the number of bypass grafts and postoperative cardiac function.

Interestingly, best results were found if the bone marrow cells were applicated via the aortic root. In these patients, an absolute 14% increase of their LVEF and a 10 mm decrease of their LVEDD six months after the operation was seen. However, intramyocardial injection did not lead to a change of LVEF at all, and LVEDD decreased only 5 mm.

Unfortunately, only four patients received the stem cell via the aortic root as a "last option" when other applications were not possible. Nevertheless, it seems to be particularly effective. This might be due to the site of cellular action concerning the infarcted area. Cells reach the periphery of the scar via open coronary arteries and collaterals after intra-aortic injection. In contrast, direct intracardiac injection and injection via bypass grafts to infarct vessels result in the application of cells into more or less central parts of the infarcted area. However, the central scar is a less sufficient nutritive environment for the transplanted cells compared to the border of the infarcted area which might be crucial for cell transformation. The promising role of intra-aortic application should be further verified.

Significant improvements of scar proportions of transplanted infarct segments were detected by Ga-MRI. Despite a positive impact of bone marrow cells on infarction size was found in experimental[3] as well as clinical[11] studies, previously, it is still under debate, and its mechanism is not fully understood. A limitation of our study is the low sensitivity of the applied scar quantification method. Changes of up to 25% of scar proportion in a segment can still have the same score, for instance. Other attempts to quantify the infarcted area, for example, encircling the scar area, have failed due to the disseminated appearance of the nonvital tissue. More work needs to be done to refine this method.

In conclusion, autologous transplantation of unselected BMC concomitant with CABG is safe and feasible. It leads to a significant improvement of ventricular function and geometry compared to isolated CABG. Moreover, it reduces myocardial scar proportion and heart failure symptoms.

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