Cardiac Effects of Experimental Intravenous Bone Marrow Cell Transplantation after Myocardial Infarction

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Objectives: Chronic heart failure after myocardial infarction is still a serious problem without a fundamental therapy. Direct intramyocardial transplantation of bone marrow cells (BMC) is promising but difficult to perform. Therefore, cardiac effect of experimental intravenous application of BMC after myocardial infarction (MI) is evaluated.

Methods: 20 Lewis rats underwent suture ligation of the LAD. One month after the MI, they were randomized to receive either intravenous Lewis-BMC or saline injection. Hearts were explanted and histologically examined another month later. Transthoracic echocardiography was performed before MI and intravenous injection as well as before explantation.

Results: BMC transplanted animals developed less cartilaginous metaplasia (BMC-group: 30% vs Control-group: 50%, p <0.01). Moreover, systolic thickness of the interventricular septum (IVSs) increased significantly in the BMC-group only: pre-Tx 1.4 ± 0.5 mm vs post-Tx 2.3 ± 0.5 mm, p = 0.02; whereas, systolic left ventricular posterior wall diameter (LVPWD) increased in the control groups only: pre-Tx 2.6 ± 0.5 mm vs post-Tx 3.4 ± 0.8 mm, p = 0.04. BMC transplantation showed a tendency towards a smaller infarct area (BMC group, 11% vs. Control group, 13%; p = 0.07) and increases in LVEF and FS after an intravenous injection (p = 0.08).

Conclusion: Intravenous BMC-Tx led to less calcifying remodelling and a compensatory hypertrophy within the infarction area that probably contributes to functional recovery.

Keywords: bone marrow cell transplantation, experimental myocardial infarction

Introduction

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

Since it is known that adult bone marrow stem cells are able to differentiate into cardiomyocytes,2) the concept of intracardiac transplantation of autologous bone marrow cells was experimentally3,4) and clinically5,6) evaluated.
Functional geometric and morphologic parameters of the diseased hearts were more or less positively influenced, depending on the selected cell types and application procedures. Different, complex preselection, cultivation and application procedures were commonly performed before transplantation. In order to simplify the cell harvest and preparation we transplanted unselected, noncultivated bone marrow cells in a previous study. We found it to be safe and adjunct with CABG it led to an improvement in ventricular geometry and function, reduced myocardial scar proportion, as well as heart failure symptoms. This time, we sought to simplify the way of transplantation by injecting the BMC intravenously because catheter-based and surgical applications are complex, invasive procedures with potential risks.

**Methods**

**Study design**

A prospective, randomized and blinded animal study with paired samples was conducted in accordance with guidelines for the care and use of laboratory animals at our institution and with approval by the local animal protection commission.

**Animal preparation, myocardial infarction and i.v. injection**

Twenty Lewis rats with a mean body weight of 347g (median 348; range 315–400; SD 21) were obtained from a single breeding colony (Harlan Winkelmann, Borchten). Animals had free access to standard laboratory food and water ad libitum. Twelve hours of light per day were provided. All rats underwent surgical ligation of the LAD to produce myocardial infarction. They were anesthetized with xylazine (3.7 mg/kg i.p.) and ketamine (66.5 mg/kg i.p). After endotracheal intubation and ventilation, a left lateral thoracotomy was performed. The pericardium was opened, and the left coronary artery was proximally suture ligated with 8-0 Prolene. Myocardial ischemia could always be visualized as well as akinesia. The chest was closed and air-aspirated. All rats resumed spontaneous breathing after mechanical ventilation was finished. There were no operative deaths. Four weeks later, they were randomized to receive either BMC or pure saline. A laparotomy was performed in all animals, and treatment solutions were injected directly into the inferior vena cava. An Intravenous injection, instead of an intramyocardial, of the BMC was performed to simplify cell application and thereby lower the risk of application-related complications.

**BMC harvest**

Both Femora of donor Lewis rats were collected after i.v. heparinization (40 IE/100g body weight). Both ends of bones were cut away from the diaphysis. Hyaluronidase (100 µl) was injected into the bone, and bone marrow plugs were hydrostatically expelled after 3 minutes with 0.9% saline. Marrow plugs were then washed in a heparin-saline solution, filtrated, and afterwards centrifuged (500G, 10 minutes). A mean of 50 × 10⁶ BMC cells were resuspended in 150 µl 0.9% saline and immediately transplanted.

**Echocardiographic examination**

Echocardiographic studies were performed before myocardial infarction, i.v. injection and explantation by a VIVID 7 dimension system (General Electric-Vingmed Ultrasound, Horton Norway). Images were obtained by a 10S transducer (5.5–12.0 MHz) with high temporal and spatial resolution. Rats underwent the same intraperitoneal anesthesia as for the surgery which was maintained throughout the echocardiographic examination. The transducer was placed directly on the shaved chest wall. A complete 2-dimensional and M-mode echocardiogram according to standards of the American Society of Echocardiography was performed.

**Pathohistological examination**

Explanted hearts were cut at short axis into 6–8 pieces. These were fixed in formalin, embedded in paraffin and sectioned into 2 µm slices. Common staining was performed with hematoxylin and eosin as previously applied in murine myocardial infarction model. In order to calculate infarct proportion one histological slice per paraffin slice was scanned and digitalized. Afterwards, the following Areas (mm²) were planimetrically determined with the Fiji-program based on ImageJ:

- AH - area of the whole heart (within epicardial circumference)
- AR - area of the right cavum (within endocardial circumference)
- AL - area of the left cavum (within endocardial circumference)
- AI - area of myocardial infarction

Then heart volume (VH) and infarct volume (VI) were calculated with the slice number (1-n) and slice thickness (TS) with the following formulas:
VH = (AH1-AR1-AL1) × TS1 +...+(AHn-ARn-ALn) × TSn
Vl = A1 × TS1 +...+ An × TSn
Myocardial scar proportion (MSP) (%) resulted from (Vl/VH) × 100.

Immunohistochemical analysis to detect the cartilaginous metaplasias was performed using staining machine (BenchMarc®, Firma Roche Ventana) as well as monoclonal mice S100 antibodies (clone 15E2E2) from Bio-Genix, Germany.

Statistics
Statistical analysis was done using Prism 5 (GraphPad Inc., La Jolla, USA). Values are expressed as mean ± standard deviation (SD) unless indicated otherwise. Paired samples of metric data were compared by t-test or Wilcoxon test, depending on the presence of Gaussian distribution. The level of significance was $\alpha \leq 0.05$.

Results
Myocardial infarction led to a significant reduction in LVEF (70 ± 6 % vs 40 ± 8 %, $p < 0.0001$), FS (35 ± 5 % vs 17 ± 4 %, $p < 0.0001$) and IVSs (2.3 ± 0 mm vs 1.6 ± 1.0 mm, $p < 0.01$) as well as to an increase of LVEDD (7.0 ± 1.0 mm vs 9.0 ± 1.0 mm, $p < 0.0001$) in all animals, compared to the baseline. LVPWs did not change at all (2.0 ± 0.0 mm vs 2.0 ± 0.0 mm, ns). BMC group showed a tendency towards an increase in LVEF and FS after an intravenous injection. Mean LVEDD increased only in the control group, but without significance. Systolic thickness of interventricular septum increased only significantly in the BMC-group (IVS: pre-Tx 1.4 ± 0.5 mm vs post-Tx 2.3 ± 0.5 mm, $p = 0.02$, see Fig. 1 whereas systolic thickness of left ventricular posterior wall increased only significantly in the control group (LVPWs: pre-Tx 2.6 ± 0.5 mm vs post-Tx 3.4 ± 0.8 mm, $p = 0.04$, see Fig. 2.) Direct comparison of the change $\Delta$ (preTx-postTx) between BMC and saline group did not show a significant difference in LVEF and FS as well as in LVEDD. Echocardiographic measurements, before and one month after BMC-Tx or saline injection, are summarized in Table 1. Explanted hearts of BMC transplanted animals showed tendency toward less size of the myocardial scar proportion (MSP Tx-group: 11 ± 1 % vs MSP Controls: 13 ± 2 %, $p = 0.07$). Cartilaginous metaplasias (Fig. 3), which had been previously described after experimental myocardial infarction as a common post infarct remodelling phenomena, were significantly fewer in BMC transplanted hearts (3/10) than in those of control animals (5/10) ($p < 0.01$).

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 recently failed to improve heart function after myocardial infarction. However, experimental results showed the ability of cells from the bone marrow to differentiate into cardiomyocytes. 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. Interventionsal attempts focused on early treatment of patients after acute myocardial infarction, whereas surgery concentrated on chronic heart failure. Different bone marrow cells types have been used commonly after selection and cultivation. 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. Furthermore, preselection of bone marrow cells might exclude other potentially effective cells and prevent harvest and transplantation during the same procedure. Therefore, we decided to transplant unselected and uncultivated bone marrow cells. In a previous study, we performed transplantation of those cells during coronary bypass procedures and compared it with isolated coronary surgery. No complications occurred, and cardiac geometry, function, scar proportion and the NYHA class improved significantly six months after the operation in the transplant group only, but not in the matched control group. Thereby, we simplified the cell preparation and showed its effectiveness. In the present study, we intended to simplify the way of application since previously performed interventional as well as surgical application carry significantly potential risks like cardiovascular injuries or wound healing problems. Therefore, we injected the unexpanded BM cells intravenously and avoided heart catheterization or thoracotomy. To our knowledge this approach to treat ICM was unique and not evaluated before. BMC transplanted animals showed a tendency towards better functional recovery after myocardial infarction and less remodelling. Particularly, cartilaginous metaplasias, which were previously described after experimental myocardial infarction as a common postinfarct remodelling phenomena, were significantly more detected in the control animals without BMC-Tx. Interestingly, only transplanted animals were able to show a significant hypertrophic response within the infarcted myocardium represented by the IVSs increase. Control animals showed only a significant hypertrophy of the non-ischemic posterior wall which probably led to slightly improved functional echocardiographic findings one month after saline injection and partially compensates the dysfunction of the anteroseptal segment after LAD ligation. In contrast, BMC transplanted hearts showed only a significant hypertrophic reaction of the septum which might have been compensation enough without a need for posterior hypertrophy.

As suspected before it is not mandatory to transplant the stem cells directly into the scar to get a positive cardiac effect. Intracoronary, intraaortic and even intravenous applications can be effective and might be superior to direct injections into the scar. This might be due to the side of cellular action concerning the scar. Cells reach the periphery of the scar via open coronary arteries and collaterals after intracoronary, intraaortic and intravenous applications. In contrast, direct intracardiac injection and injection via bypass grafts to infarct vessels result in application of cells into, more or less, central parts of the scar. However, the central scar is in a less sufficient nutritive environment for transplanted cells compared to the scar border, which might be important for cell transformation. One might be suspicious if intravenously given BMC can pass pulmonary capillaries and reach the arterial system and particularly coronary arteries. However, even radioactive labeled endothelial progenitor cells had been given intravenously and could be detected afterwards in different organs including the myocardium hours later. Crucial for an effective stem cell-mediated repair of infarcted myocardium seems to be several

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Before iv injection (mean/SD/min/max)</th>
<th>1 month after iv injection (mean/SD/min/max)</th>
<th>p-value</th>
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<tbody>
<tr>
<td><strong>BMC-TX</strong></td>
<td></td>
<td></td>
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<tr>
<td>LVEF – %</td>
<td>40/8/26/49</td>
<td>48/14/30/65</td>
<td>0.08</td>
</tr>
<tr>
<td>FS – %</td>
<td>17/4/10/22</td>
<td>22/8/12/32</td>
<td>0.08</td>
</tr>
<tr>
<td>IVSs – mm</td>
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<td>2.3/0.5/2.0/3.0</td>
<td>0.02</td>
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<td>LVEDD – mm</td>
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<td>8.6/1.1/7.0/10.0</td>
<td>0.73</td>
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<tr>
<td>LVPWs – mm</td>
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<td>2.8/0.4/2.0/3.0</td>
<td>0.77</td>
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<td><strong>Controls</strong></td>
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<tr>
<td>LVEF – %</td>
<td>40/9/25/50</td>
<td>46/8/32/57</td>
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<tr>
<td>FS – %</td>
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<td>20/4/13/26</td>
<td>0.22</td>
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<tr>
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<td>2.2/0.4/2.0/3.0</td>
<td>0.20</td>
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<td>9.4/1.5/7.0/12.0</td>
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<td>3.4/0.8/2.0/5.0</td>
<td>0.04</td>
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</table>
signalling factors like SDF-1, TNF-α or VEGF, which are involved in stem cell mobilization and chemotaxis, as well as in cardio- and angiogenesis.  

The promising role of intravenous application of BMC to treat ischemic cardiomyopathy should be further verified. A randomized, clinical study, including a sufficient number of patients receiving BMC in this way, should be conducted.

A limitation of the present study is that we did not try to detect the BMC in the myocardium. Moreover, infarct size quantification could be more precisely and longer follow up intervals would be interesting.

In conclusion, intravenous application of BMC after experimental myocardial infarction has positive cardiac effects and is easy to perform. It leads to less calcifying remodelling and a compensatory hypertrophy within the infarction area that probably contributes to functional recovery.

References