Myocardial regeneration by transplantation of modified endothelial progenitor cells expressing SDF-1 in a rat model

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Abstract

Cell based therapy has been shown to attenuate myocardial dysfunction after myocardial infarction (MI) in different acute and chronic animal models. It has been further shown that stromal-cell derived factor-1α (SDF-1α) facilitates proliferation and migration of endogenous progenitor cells into injured tissue. The aim of the present study was to investigate the role of exogenously applied and endogenously mobilized cells in a regenerative strategy for MI therapy. Lentivirally SDF-1α-infected endothelial progenitor cells (EPCs) were injected after 90 min. of ligation and reperfusion of the left anterior descending artery (LAD) intramyocardial and intracoronary using a new rodent catheter system. Eight weeks after transplantation, echocardiography and isolated heart studies revealed a significant improvement of LV function after intramyocardial application of lentiviral with SDF-1 infected EPCs compared to medium control. Intracoronary application of cells did not lead to significant differences compared to medium injected control hearts. Histology showed a significantly elevated rate of apoptotic cells and augmented proliferation after transplantation of EPCs and EPCs + SDF-1α in infarcted myocardium. In addition, a significant increased density of CD31+ vessel structures, a lower collagen content and higher numbers of inflammatory cells after transplantation of SDF-1 transgenic cells were detectable. Intramyocardial application of lentiviral-infected EPCs is associated with a significant improvement of myocardial function after infarction, in contrast to an intracoronary application. Histological results revealed a significant augmentation of neovascularization, lower collagen content, higher numbers of inflammatory cells and remarkable alterations of apoptotic/proliferative processes in infarcted areas after cell transplantation.

Keywords: myocardial infarction – cell transplantation – endothelial progenitor cells – chemokines – heart regeneration

Introduction

Cell transplantation as well as gene therapies are promising strategies in the treatment of cardiac dysfunction after MI. Several studies demonstrated improvement of myocardial function after transplantation of various cell types [1–7]. Recently, we and other groups could demonstrate that different cell types act via different mechanisms to improve myocardial contractility [5–8]. One important point seems to be the amount of vessel structures in infarcted areas. Evidence for a significantly higher amount of vessels in infarcted areas pointed towards neovascularization being responsible for a better contractile function [5]. However, because most implanted cells were lost to ischaemia, apoptosis and inflammatory alterations, the aim of this study was to...
improve the function of remaining transplanted cells to augment neo-
vascularization and nutrition of damaged myocardium. Cell engineer-
ing is another possibility to optimize cellular function after trans-
plantation. Nakamura et al. could demonstrate a significant
improvement of cell survival and ventricular function after infection
of transplanted cells by pre-treatment with antiapoptotic Bcl-2 gene [9].
Additional studies dealing with infection of cells with different growth
factors could underline, that genetical alterations could improve the
effectiveness of cell transplantation. Yang et al. established a method
to transfect vascular endothelial factor (VEGF) gene into mesenchy-
mal stem cells and showed a better restoration of heart function and
angiogenesis after MI [10]. Even the SDF-1 is shown to be an effec-
tive and important chemokine inducing neovascularization even in
infarcted areas [11]. The production of SDF-1 is transiently increased
after tissue ischaemia and initiates the migration of progenitor cells.
Furthermore, it has a major part in embryological hematopoiesis,
vessels, and cardiogenesis.

In the present study we transplanted cells lentivirally infected with
the chemokine SDF-1 to improve neovascularization by augmented
and prolonged SDF-1 production after cell transplantation as well as
to mobilize host EPC to the infarcted areas. The aim of our study was
to combine an exogenous and endogenous cell therapy.

Another goal of the study presented herein was to determine, if an
intracoronary application in a rat model is feasible and comparable to
an intramyocardial injection of transplanted cells. Furthermore, we
chose an ischaemia/reperfusion model (90 min. of ischaemia) to sim-
ulate the case of a MI, followed by a catheterization as soon as possi-
ble in a rodent model, expecting to transfer these methods to large
animals or in the future to human studies. Despite all various cell
transplantation studies the optimal cell type and delivery technique
further remains to be determined. Therefore we used a special new
rodent transcarotid balloon catheter system for the intracoronary
application of cells.

Materials and methods

Cells

The EPCs were isolated from rat spleens, which were removed from
female adult Sprague-Dawley rats (200–250 g). After mincing spleens
into small pieces mononuclear cells (MCs) were separated by repeated
pipetting and Biocoil (Biochrom AG, Berlin, Germany) density gradient
centrifugation, washed twice in PBS and counted. Finally, the MCs were
resuspended in microvascular endothelial growth medium (Promo-
Cell, Heidelberg, Germany) and plated in a T-25 culture flask coated with
10 µg/ml human fibronectin (Habor Bioproducts Inc., Indianapolis, IN,
USA). After four days non-adherent cells were removed and fresh med-
ium was added. At day seven, adherent cells were detached with trypsin,
counted, resuspended at 10⁶ cells/100 µl PBS and transplanted. On
the day before transplantation, cells were incubated with BrdU (Zymed,
Wien, Austria) as described by manufacturer. EPC were characterized by
mRNA isolation and RT-PCR of endothelial markers (von Willebrand Fac-
tor- vWF- and PECAM1, Fig. 1A). Briefly, mRNA was isolated from the
EPC after seven days in culture using Micro-mRNA Isolation Kit (Qiagen,
Hilden, Germany) and RT-PCR was performed with Omniscript kit (Gia-
gen). Following oligonucleotide primers specific were used for rat vWF
cDNA (forward primer: 5′-AAGCTCTGGGTAAGACACGTCG-3′ and reverse
primer: 5′-CACCAGAGATTTGACGTCCTTT-3′) and PECAM1 cDNA (for-
ward primer: 5′-TACGTCACTGTCGAGTCTCT-3′ and reverse primer:
5′-GAGCTGTTTTTCTTCTATGAT-3′). Rat GAPDH cDNA served as house-
keeping gene and was amplified in parallel with the gene of interest (for-
ward primer: 5′-CTTGAGACTTTCAACAGCACTCC-3′, and reverse primer:
5′-TACCGAATAAGCTGCCTACAA-3′).

Lentiviral infection of cells

The HEK 239T cells were cultured in DMEM containing 4.5 g/l glucose,
10% foetal calf serum (FCS), 2 mMol/l L-glutamine, 100 U/ml penicillin
and 100 µg/ml streptomycin (PAa, Cölbe, Germany). The SDF-1 gene
expression vector (pRR-l-cpp-CMV.SDF-1a, LV.SDF-1a) was con-
structed by ligation of SDF-1a cDNA into SMAI digested pRR-l-cpp-
CMV-PreSIN [12–14]. An expression vector containing the GFP cDNA
insert was constructed as a positive control and both expression plas-
mids were sequence verified before use. Lentivirus (LV) expression and
helper vectors were cotransfected into HEK293T cells by the calcium
phosphate method, medium collected at 24 and 48 hrs and virus titres
determined as described by Bot et al. [14]. Viral integrants were deter-
mined by qPCR analysis (forward primer: GTGCAAGCAGAAGAACATTTGG,
reverse primer: CCCAGAAGTGATGAGTGGAA, primer stocks were
aliquoted and stored at −20°C until further use. Prior viral infection
EPCs were grown in Microvascular endothelial growth medium MV2
(PromoCell). On the day of infection cells were rinsed with PBS and
medium was changed to DMEM with 10% FCS. Infection was
performed in presence of 10 µg/ml diethylaminoethyl dextran at 30°C.
After 24 hr, cells were washed with PBS and were resuspended again
in MV2 medium. Control infections with virus carrying cDNA encoding
GFP have shown at least 90% efficiency. SDF-1 concentration in
cultured cells was evaluated by ELISA, which was performed from
serum or cell medium with Quantikine Mouse CXCL12/SDF-1 Immuno-
assay (R&D Systems, Wiesbaden-Nordenstadt, Germany). Cells were
transplanted 3 days after infection with LV.SDF-1a.

Generation of myocardial infarctions and cell
transplantation

Female adult Sprague-Dawley rats (200–250 g) were incubated under
general anaesthesia (1 ml/kg ketamine and 10 mg/kg xylazine, intraperi-
toneal) and positive pressure ventilation was maintained (room air sup-
plemented with oxygen) using a rodent respirator. Hearts were exposed
through a 2-cm left thoracotomy and temporal myocardial ischaemia
leading to MI was induced by suture occlusion of the LAD between the
left atrium and the right pulmonary outflow tract (7/0 polypropylene, Ethni-
cons). A 90 min. ischaemia regimen was chosen as it resulted in accept-
able intraprocedural death and significant loss of myocardial function.
After 90 min. of ligation/reperfusion of the LAD BrdU-labelled SDF-1a-
infected EPCs (10⁶, n = 8) were injected into the myocardium or intra-
coronary, respectively (n = 8). In additional animal groups we injected
non-transduced EPCs (intramyocardial (n = 8) and intracoronary
(n = 8)) and medium as control group (n = 10 per group). The areas
for injection were chosen by visual identification based on changes of
appearance due to temporal ischaemia and wall motion akinesia. Cells
were transplanted into marginal zones of the MI by syringe injection (for 1-min injection time) at three distinct but adjacent sites. After injection, the puncture holes were closed by suture, which served as a marker for the area of transplantation at follow-up thoracotomy. Intracoronary application of cells was performed by a special novel rodent catheter system that was developed in cooperation with Vimecon (Herzogenrath, Germany) for this study. After preparation of the left carotid artery the catheter system was inserted through the carotid artery and advanced to the ascending aorta, which was blocked above the coronary ostia by the inflated balloon catheter for 60 sec. During these 60 sec. cells or control medium was injected into the coronary arteries from the tip of the catheter. After reperfusion and cell application muscle layer and skin incision were closed with silk sutures. Animal experiments were approved by local authorities and complied with German animal protection law.

**Echocardiography**

Eight weeks after MI and TX (i.e. prior to transplantation) rats were anaesthetized and two-dimensional and M-mode measurements were performed with a SONOS 7500 (Philips, Hamburg, Germany) with a 15-MHz linear phased-array probe. The animals were anaesthetized and placed in the supine or lateral position. Parasternal long-axis and short-axis views were obtained, ensuring that the mitral and aortic valves and apex were well visualized. Fractional shortening was measured in long axis and orthogonally in the short axis, average of both results was used for additional analysis.

**Langendorff perfusion and assessment of the infarct size**

Eight weeks after MI and TX the rats were anaesthetized and hearts removed. Heart function was analysed applying a Langendorff apparatus with filtered Krebs-Henseleit buffer at a pressure of 100 mmHg equilibrated with 5% CO₂ and 95% O₂. A latex balloon was placed into the left ventricle to measure pressure and heart rate, using a commercially available isolated heart apparatus (Hugo Sachs Elektronik, March-Hugstetten, Germany). Perfusion pressure, LV developed pressure (LVDP; the difference between minimal diastolic pressure and maximal systolic pressure), coronary flow and heart rate were measured continuously (the last two did not show any statistically significant variance throughout the experiments). After 20 min. of stabilization, baseline values were recorded. Then preload was increased from 5 mmHg to 10, 15 and 20 mmHg. LVDP was recorded at the different preloads and developed pressure was calculated. The scar size of Left ventricle-free wall was assessed by computed planimetry of digitized images taken from hearts fixed in dissection (30 mmHg) with 10% formalin and cut into 5 μm slices.

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**Fig. 1** Infected cells and detection of transplanted cells in infarcted areas. (A) Isolated and cultured EPC were characterized by mRNA isolation and RT-PCR of endothelial markers (von Willebrand Factor – vWF- and PECAM1). (B) SDF-1 serum levels are significantly higher after lentiviral infection compared to control levels (*P < 0.05 versus control, left). Control EPCs infections using virus carrying cDNA encoding GFP have shown at least 90% efficiency (green staining for GFP, right). (C) Immunohistochemical BrDU staining of infarcted areas after cell transplantation shows positive signals for BrDU-labelled EPC-derived cells (see arrows), e.g. in capillary-like structures. Objective 40×, scale bars: 25 μm.
Identification of the cells and analysis of the infarction area

For cell identification, slides (5 μm) were stained with anti-BrdU kit (Zymed). Universal quick kit (Vector Laboratories, Axxora, Loerrach, Germany) were used with anti-CD31 antibody (Santa Cruz Biotechnology, Heidelberg, Germany) for vessel identification. For SDF-1 staining, an anti-SDF-1 antibody (Serotec, Düsseldorf, Germany) was used followed by a secondary, FITC-conjugated antibody. The nuclei undergoing apoptosis were stained with MEBSTAIN apoptosis kit II (MBL, Woburn, MA, USA). Accustain triple stain (Masson; Sigma-Aldrich, Seelze, Germany) was used to determine collagen content of infarcted regions. The stained areas were measured by computer-assisted planimetry (Diskus software, Hilgiers). Macrophages and Neutrophils were stained with alpha-naphthyl acetate esterase (Sigma-Aldrich) and naphthol AS-D chloroacetate (Sigma-Aldrich) respectively. To evidence proliferation, the sections were stained for Ki-67 (DAKO, Hamburg, Germany), followed by an anti rat-Cy3 antibody.

Data analysis

Data represent mean ± S.D. Statistical analysis was performed with Prism 4 and 5 software (Graph Pad) using unpaired Student t-test or 1-way ANOVA followed by Newman–Keuls test. Differences with P < 0.05 were considered significant.

Results

Transfection efficacy and tracking of EPC after transplantation

Control infection experiments of EPCs performed with lentivirus (LV) carrying cDNA encoding GFP 72 hrs after infection demonstrated an average efficiency of 90%, (Fig. 1A). LV-SDF1 infected EPCs efficiently secreted SDF-1 into the cell culture medium. Medium SDF-1 levels compared to control medium of non-infected EPCs were highly significantly elevated (LV-SDF-1 infected cells 379.4 ± 118 pg/ml versus 39.4 ± 6.7 pg/ml for non-infected EPC, P < 0.05, Fig. 1B).

Pre-implantation EPC that had been BrdU-labelled, 8 weeks after transplantation of EPCs were detected both at the injection site as well as incorporated in capillary-like structures after intracoronary application (Fig. 1C). Unfortunately, the Brd-U signals were very rare and it was impossible to quantify the number of injected cells refined in myocardium after the transplantation.

Remodelling and inflammation in infarcted areas

Apoptosis measured by TUNEL staining increased in the infarcted area after transplantation of SDF-1 expressing EPC, independent of their application mode (intramyocardial: LV-SDF-1-EPC TX 116.5 ± 20.7 TUNEL+ cells/mm² versus control TX 31.7 ± 11.5 TUNEL+ cells/mm²; P < 0.05, intracoronary: LV-SDF-1-EPC TX 131.3 ± 45.6 TUNEL+ cells/mm² versus control TX 19.4 ± 2.2 TUNEL+ cells/mm²; P < 0.05). When EPC only were injected into myocardium or via the coronary circulation the degree of apoptosis did not differ from medium controls (Fig. 2A).

A similar pattern is detected with the assessment of cellular proliferation in the infarcted myocardium. In both groups of EPC plus SDF-1 an augmented proliferation in the infarcted area after the application is measured (intramyocardial: LV-SDF-1-EPC TX 30.7 ± 5.6 Ki-67+ cells/mm² versus EPC 19.8 ± 4.1 Ki-67+ cells/mm²; P < 0.05, intracoronary: LV-SDF-1-EPC TX 36.4 ± 4.5 Ki-67+ cells/mm² versus control TX 14.2 ± 3.4 Ki-67+ cells/mm²; P < 0.05, Fig. 2A). EPC alone – independent of application route – did not significantly affect the proliferation compared to medium injected controls (Fig. 2B).

Interestingly the collagen content in the infarcted area measured by specific staining was significantly lower after direct intramyocardial but not after intracoronary EPC transfer when compared to the untreated control (Fig. 2B and C). The additional expression of SDF-1 did not alter the collagen content (intramyocardial: EPC TX 10.6 ± 1.2% of infarcted area versus LV-SDF-1-EPC TX 10.3 ± 1.2% of infarcted area versus control TX 14.8 ± 1.1% of infarcted area, P < 0.05, Fig. 2B). The infarct size after MI and cell or control medium injection did not differ between the groups (data not shown).

Angiogenesis and cellular inflammatory response

The effects on angiogenesis and vascular density were assessed by staining and quantification of CD31-positive vessels in the area of infarction. First looking at EPC injection alone intracoronary EPC application did not increase vessel density whereas intramyocardial injection led to quite a significant increase (intramyocardial: EPC TX 744 ± 92 vessels/mm² versus control TX 609 ± 38 vessels/mm²; P < 0.05, intracoronary: EPC TX 1142 ± 53.6 vessels/mm² versus control TX 520 ± 72.3 vessels/mm², P < 0.001. Difference between i.c. and i.m. P < 0.05). EPCs enhanced with SDF-1 infection induced a more pronounced albeit differentiated angiogenisis. SDF-1 expressing EPC injected i.c. induced a significant increase of angiogenesis (LV-SDF-1-EPC TX 1024 ± 47 vessels/mm²) compared to control (P < 0.05) although not reaching significance compared to the EPC only group. Intramyocardial injection of LV-SDF-1-EPC induced the most prominent angiogenesis response almost tripling the amount of blood vessels found in controls (LV-SDF-1-EPC TX 1523 ± 280 vessels/mm²) meeting statistical significance in comparison to EPC i.m. (P < 0.05) and medium controls (P < 0.001) but even in comparison to i.c. injection of the same cell type (P < 0.01, Fig. 3A and B).

As expected, we found an increased SDF-1 staining in the transfected EPC groups, correlating successfully to CD31 staining (Fig. 3C).

As indicators of inflammation, we assessed monocyte/neutrophil cell infiltrate in all groups by staining for specific/unspecific esterase. The most evident infiltration of monocytes within the infarcted territory was seen in the EPC plus SDF1 groups (intramyocardial: LV-SDF-1-EPC TX 182.8 ± 40.7 cells/mm² versus control TX 47.1 ± 3 cells/mm², P < 0.01; intracoronary: EPC/SDF-1 TX 146.8 ± 45.4 cells/mm² versus control TX 57.1 ± 11 cells/mm², ns). When EPC alone were injected again only the intramyocardial group reached significance compared to controls (intramyocardial: EPC TX 93.1 ± 7.4 cells/mm², P < 0.05 versus controls; intracoronary: EPC TX
Fig. 2 Apoptosis, proliferation and collagen content in infarcted areas. TUNEL staining was used to identify increased numbers of apoptotic cells (A) in with SDF-1 transfected cells treated hearts (*P < 0.05 versus control). (B) To assess proliferation, the sections were stained for Ki-67 (DAKO), followed by an anti-rat-Cy3 antibody (green; *P < 0.05 versus control). Collagen content in infarcted areas (C, D) was significantly lower after intra-myocardial transplantation of EPC and lentivirally with SDF-1 transfected EPC. Intracoronary cell application did not result in changes of collagen concentration (blue; scale bar: 100 μm; *P < 0.05 versus control).

Fig. 3 Vessel density and SDF-1 expression in infarcted areas. (A, B) Vessel density per mm² (green: CD 31 positive vessels; objective 20×; scale bar: 50 μm; *P < 0.01 versus control) was significantly increased in cell-treated hearts compared with controls. Transplantation of SDF-1 transfected cells further increased vessel density (*P < 0.05; **P < 0.01; ***P < 0.001). (C) but also the SDF-1 expression in infarcted hearts (green: SDF-1 positive staining; blue: DAPI nucleus staining; objective 40×; scale bar: 25 μm).
In sharp contrast, neutrophil influx was impaired after transplantation of LV-SDF-1-EPC but not after EPC only transfer – independent of application type (intramyocardial: EPC TX 65.2 ± 15.9 cells/mm² versus LV-SDF-1-EPC TX 39.8 ± 10.4 cells/mm² versus control TX 100.2 ± 25 cells/mm²; intracoronary: EPC TX 68.1 ± 29.9 cells/mm² versus LV-SDF-1-EPC TX 30.1 ± 4.2 cells/mm² versus control TX 76.2 ± 15.4 cells/mm², P < 0.05; Fig. 4).

Cardiac function

Although we were able to demonstrate a differentiated tissue response the key performance indicator of the heart is the assessment of its contractile function. To assess cardiac function, we performed two-dimensional echocardiography (fractional shortening (FS) in %) before and after MI/cell transplantation (Fig. 5A). Eight weeks after MI echocardiographic analysis revealed a significantly impaired LV function in the medium control groups (baseline FS: 48.3 ± 5.9%, medium control i.c.: 36.7 ± 6.8% and medium control i.m.: 33.3 ± 5.9%; P < 0.001 compared to baseline; ns between controls). The LV FS deteriorated by 11.6% and 14.9% respectively. Transplantation of EPC improved LV FS by 2.95% comparing intracoronary application (EPC i.c. FS: 39.6 ± 6.0%; ns compared to medium control i.c.). In the intramyocardial application group the FS increased by 7.4% compared to medium control group but again not reaching statistical significance (EPC i.m. FS: 43.0 ± 5.7%; ns compared to medium control i.m.). Transplantation of SDF-1 infected EPC led to a LV FS increase of 6.4% compared to controls (LV-SDF-1-EPC i.c. 42.2 ± 5.1%; P < 0.05 compared to medium control i.m.). Although the LV-SDF-1-EPC transplanted hearts showed a trend towards better contractility there was no significant difference between EPC and EPC/SDF-1 groups regarding this parameter.

For further quantification of LV function, we performed isolated heart studies with retrograde perfusion according to Langendorff. LVDP was significantly augmented in hearts after intramyocardial transplantation of EPCs or SDF-1 infected EPCs (LVDP: after EPC TX 137 ± 24.9 mmHg versus LV-SDF-1-EPC 144.1 ± 16.2 mmHg versus control 103.4 ± 19.2 mmHg, P < 0.05). Intracoronary injection showed a trend towards improved LVDP but did not reach statistical significance (LVDP: after EPC-TX 128.9 ± 14.7 mmHg versus LV-SDF-1-EPC 121.1 ± 24.5 mmHg versus control 111.8 ± 19.4 mmHg, P < 0.05, Fig. 5B).

Discussion

Recent studies have shown that cardiomyocyte regeneration is a process taking place throughout our entire life, at the age of 55

![Cellular inflammatory response in infarcted areas. (A, C) Number of monocytes (yellow-brown) were increased in infarcted areas after direct intramyocardial injection and after intracoronary application but particularly when cells were also expressing SDF-1, while neutrophils (B,C) were decreased in cell-treated hearts (red; objective 40×; scale bar: 25 μm; *P < 0.05 versus control).](image)
45% of all cardiomyocytes have been generated after adolescence and 0.45–1% are regenerated every year [15]. Cell therapy for cardiac repair has been propagated not only in various permutations in animal models but also clinical trials. Especially the latter mostly used bone marrow-derived mononuclear cells (BMCs) with meta-analyses of these trials demonstrating an average improvement of LV ejection fraction around 4% [16, 17]. Other cell populations that have been studied are haematopoietic and mesenchymal progenitor cells. Recent interest has also been focussed on resident cardiac progenitor cells. These resident cells are expected to differentiate into contractile cardiomyocytes although the other types of cells are thought to be more involved in influencing the myocardial environment facilitating remodelling, angiogenesis and inflammatory response [18].

On the other hand gene therapy of the heart is a promising approach to interfere with pathophysiological processes. However it has been limited by problems of delivery, efficacy, cell specificity and side effects [19]. Experimental animal studies have shown to promote angiogenesis and attenuate apoptosis. A recently published study analysed the response to intramyocardial injection of adenoviral vectors encoding SDF-1 in an animal model of MI demonstrating changes in collagen expression as well as TGF-β1, TIMP-1 and TIMP-2 [20]. Gene therapy appears to be an ideal partner of cell therapy allowing specific alteration of transplanted cells, using the cells as a vehicle and limiting systemic side effects. Rong et al. demonstrated that transplantation of heart cells overexpressing human growth hormone led to induced angiogenesis and diminished apoptosis [21]. Also combined growth hormone gene transfer and cell transplantation provided an effective strategy for improving post-infarction ventricular function. To transfect cells with different hormones, cytokines and growth factors several techniques have been established [22-25]. Genetically altered cells or cells pre-conditioned with growth factors seem to have an additional impact on improvement of myocardial function [26-28]. Yang et al. could show in a rat model that transplantation of mesenchymal stem cells (MSCs), liposomally transfected with VEGF gene ameliorated myocardial perfusion and restored heart function more potently than either cellular or gene therapy alone [10]. In another study by Liu et al. [29] mesenchymal stem cells were transfected by an adenoviral vector with angiogenin, the angiogenin modified stem cells then enhanced the tolerance of engrafted cells to hypoxia injury in vitro and improved their viability in infarcted hearts, thus helping preserve the LV contractile function and attenuate LV remodelling through vasculogenesis. Sun et al. [30] used human angiopoietin-1 (hAng1)-modified MSCs to treat acute MI in rats. The hAng1 gene was transfected into cultured rat MSCs also using an adenoviral vector. The results indicated that hAng1-modified MSCs improved heart function and angiogenic effects. Despite of the limitations shown in animal and clinical studies cell and gene therapies remain potential new treatments for cardiovascular diseases [30].

In most studies performed with BMCs neovascularization was the key mechanism to achieve cardiac repair after MI while direct cardiomyogenesis is not likely to be expected from this strategy [8]. Inflammatory processes triggered by cell transplantation potentially contribute to and modify remodelling processes [6]. Modulation of inflammatory processes as well as paracrine effects by transplanted cells have also been suggested to play an important role in maintaining cardiac function. Dai Y et al. [28] concluded that mobilized progenitor cells are highly cytoprotective and carry protective genes responsible for cardiac repair.

The CXC chemokine SDF-1 has been shown to play a major role in embryological hemato-, vasculo- and cardiogenesis and is transiently increased after tissue ischaemia and initiates the migration of progenitor cells. Recently [5], we could demonstrate that injection of SDF-1 alone and combined with EPC in a MI model is able to augment neo-vascularization and seems to be associated with attenuated heart function. Also Elmadbouh et al. [11] transfected human stromal cell derived factor (SDF-1) gene into skeletal myoblasts and transplanted these cells to establish a transient SDF-1 gradient to favour extra-cardiac stem cell translocation into infarcted heart. They could show that ex vivo SDF-1 transgene delivery promotes stem and progenitor cell migration to the heart and enhances angiomyogenesis in the infarcted areas. Recently it has also been demonstrated that SDF-1 expression...
in the heart enhances the homing of bone marrow derived stem cells to the area of infarction [31].

The aim of the present study was to combine exogenous stem cell therapy with enhanced mobilization of endogenous progenitor cells in the treatment of MI. The model of MI sought to simulate the condition of temporary coronary occlusion with reestablishment of coronary flow within less than 2 hrs and cell transplantation via the re-opened coronary or direct myocardial injection. We transplanted SDF-1 expressing transgenic EPCs to mobilize endogenous progenitor cells and augment the number of newly formed vessel structures as well as the expression of SDF-1 in the infarcted areas. A lentiviral construct encoding SDF-1 was chosen for gene delivery with control experiments demonstrating an EPC infection rate of 90% before transplantation. SDF-1-ELISA of the supernatant in cell cultures also showed highly significant elevation of SDF-1 production. SDF-1 relies on a concentration gradient to attract progenitor cells to the site of injury in this study, accordingly systemic SDF-1 concentration in peripheral blood samples from defined time points after cell transplantation did not differ between groups (data not shown). SDF-1 is also thought to facilitate migration of cells across the endothelial barrier therefore supporting endogenous circulating stem cell populations in reaching the infarcted myocardium [31].

Table 1 summarizes the results of the present study displaying changes relative to controls and levels of significance 8 weeks after cell transplantation. Temporary myocardial ischaemia and reperfusion led to a significant impairment of LV function in the control groups. Cell transplantation especially with intramyocardial application and in combination with SDF-1 expressing EPC led to an increase of LV function. This correlates best with the findings for neovascularization, which was also most pronounced in the groups performed with intramyocardial application as well as SDF-1 expressing EPC. Looking at remodelling parameters the infarcted areas in the groups of intramyocardial cell injection contained less collagen but more blood vessels. Because this effect was independent of SDF-1 expression it might be associated with a different retention of injected EPC via an intramyocardial route. However the absolute number of residual and remaining stem cells could not be determined therefore this hypothesis would remain speculative. Another parameter of remodelling was apoptosis which was clearly stimulated by SDF-1 expression. Although the study was not designed to describe the cell population undergoing apoptosis it has been described elsewhere that anti-apoptotic effects are desirable features of stem cell therapy [32]. On the other hand the SDF-1 expressing implanted cells induced proliferation in the infarcted area. Again, the type of proliferating cells was not identified but studies performed with indirect methods to increase SDF-1 secretion demonstrated an increase in c-Kit(+) and CD34(+) stem cells suggesting a certain portion of cardiac progenitor cells and endogenous bone marrow-derived cells [33–35].

At the time-point of experiment termination both groups of SDF-1 expressing EPC were associated with lower neutrophils but higher monocyte content in the infarcted territory. In agreement to other studies, a part of the monocyte population might represent migrated bone marrow derived MCs.

The other aim of our study was to compare different application methods. Therefore, we created a novel intracarotid rodent balloon catheter system for an intracoronary cell application. Although the intramyocardial injection appeared to be more efficient in the groups performed with EPCs only the additional expression of SDF-1 somewhat compensated for that. One possible mechanism explaining the different results of intracoronary cell application might be that SDF-1 facilitated transendothelial migration for some of the remaining transplanted as well as endogenous cells as demonstrated through a CXCR4 receptor mediated mechanism elsewhere [36].

Limitations of the study lie in particular in the infarction model chosen that led to only relatively moderate degrees of MI, even in the group most affected the mean fractional shortening was still 33.3% or had - compared to baseline - decreased by 14.9%. Although this model of infarction sought to emulate clinical circumstances with infarction and early reperfusion the moderate infarction size might have blurred the differences between the EPC groups. This and the sample size of 8–12 animals per group also meant that although obvious trends of improvement were seen they did not reach statistical significance in all groups. An additional limitation is the single time-point at which the experiment was ended, the persistent level of inflammatory response and proliferation/apoptosis detected in some groups could be interpreted as continuing remodeling that has not yet reached its conclusion. Although we postulate that SDF-1 supports mobilization of endogenous stem cells from bone marrow, an effect that has also been published elsewhere [31], the amount of mobilized stem cells was not quantified in the present study. As with most small animal models the potential effect of concomitant pharmacotherapy used in clinical medicine has not been taken into consideration. It has to be further acknowledged that the endpoints of our study were rather functional parameters than clini-

### Table 1

|                  | Macrophages | Monocytes | Apoptosis | Proliferation | Collagen | Anglogenesis | Echo (FS in %) | LVDP |
|------------------|-------------|-----------|-----------|--------------|----------|--------------|---------------|------|
| EPC i.m.         | =           | =         | =         | =            | (↑)      | (↓↓)         | (↑↑↑↑↑↑)      | =    |
| EPC/SDF1 i.c.    | ↓ (↑)       | ↑↑         | ↑         | =            | ↑         | ↑↑↑↑↑↑↑↑↑     | (↑↑↑↑↑↑↑↑↑↑)   | ↑    |
| EPC i.c.         | ↓           | ↑         | ↑↑         | (↑)          | ↓         | ↑↑↑↑↑↑↑↑     | (↑↑)          | ↑    |
| EPC/SDF1 i.m.    | ↓           | ↑         | ↑↑         | (↑)          | ↓         | ↑↑↑↑↑↑↑↑     | (↑↑↑↑↑↑↑↑)    | ↑    |

↑ or ↓: P < 0.05; ↑↑ or ↓↓↓: P < 0.01; ↑↑↑ or ↓↓↓↓: P < 0.001; (↑↑): strong tendency but non significant; =: no relevant changes.
Infection of EPC with SDF-1 is feasible and leads to SDF-1 secretion. LV-SDF-1-EPC can be transplanted via coronary route or through intramyocardial injection and persist in tissue. Although an intramyocardial transplantation of EPC seems to be more successful than intracoronary injection, the addition of SDF-1 expression seems to partially compensate for that effect. SDF-1 expression changes the tissue response and it remains to be determined which of these responses are desirable for improvement of LV function and remodelling. Additional studies are required to evaluate the effect of transgenic SDF-1 expression through stem cells in respect to infarct size, time course, animal model and concomitant medication or chemokine application.

**Conclusion**

Infection of EPC with SDF-1 is feasible and leads to SDF-1 secretion. LV-SDF-1-EPC can be transplanted via coronary route or through intramyocardial injection and persist in tissue. Although an intramyocardial transplantation of EPC seems to be more successful than intracoronary injection, the addition of SDF-1 expression seems to partially compensate for that effect. SDF-1 expression changes the tissue response and it remains to be determined which of these responses are desirable for improvement of LV function and remodelling. Additional studies are required to evaluate the effect of transgenic SDF-1 expression through stem cells in respect to infarct size, time course, animal model and concomitant medication or chemokine application.

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**Disclosures**

None.

**References**

1. Tomita S, Li RK, Weisel RD et al. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation*. 1999; 100: 11247–56.
2. Fuchs S, Baffour R, Zhou YF, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol*. 2001; 37: 1726–32.
3. Skobel E, Schuh A, Schwarz ER, et al. Transplantation of foetal cardiomyocytes into infarcted rat hearts results in long-term functional improvement. *Tissue Eng*. 2004; 10: 849–64.
4. Merx MW, Zernecke A, Liehn EA, et al. Transplantation of human umbilical vein endothelial cells improves left ventricular function in a rat model of myocardial infarction. *Basic Res Cardiol*. 2005; 100: 208–16.
5. Schuh A, Liehn EA, Sasse A, et al. Transplantation of endothelial progenitor cells improves neovascularization and left ventricular function after myocardial infarction in a rat model. *Basic Res Cardiol*. 2008; 103: 69–77.
6. Schuh A, Liehn EA, Sasse A, et al. Improved left ventricular function after transplantation of microspheres and fibroblasts in a rat model of myocardial infarction. *Basic Res Cardiol*. 2009; 104: 403–11.
7. Schuh A, Breuer S, Al Dashi R, et al. Administration of vascular endothelial growth factor adjunctive to foetal cardiomyocyte transplantation and improvement of cardiac function in the rat model. *J Cardiovasc Pharmacol Ther*. 2005; 10: 55–66.
8. Dubois C, Liu X, Claus P, et al. Differential effects of progenitor cell populations on left ventricular remodeling and myocardial neovascularization after myocardial infarction. *J Am Coll Cardiol*. 2010; 55: 2232–43.
9. Nakamura Y, Yasuda T, Weisel RD, et al. Enhanced cell transplantation: preventing apoptosis increases cell survival and ventricular function. *Am J Physiol Heart Circ Physiol*. 2006; 291: H939–47.
10. Yang JF, Zhou WW, Tang T, et al. Effects of myocardial transplantation of mesenchymal stem cells transplanted with vascular endothelial factor gene on improvement of heart function and angiogenesis after myocardial infarction: experiment with rats. *Zhonghua Yi Xue Za Zhi*. 2006; 86: 1027–34.
11. Elmadbouh I, Haider HKh, Jiang S, et al. Ex vivo delivered stromal cell-derived factor-1alpha promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium. *J Mol Cell Cardiol*. 2007; 42: 792–803.
12. Seppen J, Rijnberg M, Cooreman MP, et al. Lentiviral vectors for efficient transduction of isolated primary quiescent hepatocytes. *J Hepatol*. 2002; 36: 459–65.
13. Zufferey R, Dull T, Mandel RJ, et al. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J Virol*. 1998; 72: 9873–80.
14. Bot I, Guo J, Van Eck M, et al. Lentiviral shRNA silencing of murine bone marrow cell CCR2 leads to persistent knockdown of CCR2 function in vivo. *Blood*. 2005; 106: 1147–53.
15. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. *Science*. 2009; 324: 98–110.
16. Chavakis E, Koyanagi M, Dimmeler S. Enhancing the outcome of cell therapy for cardiac repair: progress from bench to bedside and back. *Circulation*. 2010; 121: 325–35.
17. Lippinski MJ, Biondi-Zoccai GG, Abbate A, et al. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: a collaborative systematic review and meta-analysis of controlled clinical trials. *J Am Coll Cardiol*. 2007; 50: 1761–7.
18. Tongers J, Losordo DW, Landmesser U. Stem and progenitor cell-based therapy in ischemic heart disease: promise, uncertainties, and challenges. *Eur Heart J*. 2011; 32: 1197–206.
19. Ding Z, Fach C, Sasse A, et al. A minimally invasive approach for efficient gene delivery to rodent hearts. *Gene Ther*. 2004; 11: 260–5.
20. Tang J, Wang J, Song H, et al. Adenovirus-mediated stromal cell-derived factor-1 alpha gene transfer improves cardiac structure and function after experimental myocardial infarction through angiogenic and antifibrotic actions. *Mol Biol Rep*. 2010; 37: 1957–69.
21. Rong SL, Lu YX, Liao YH, et al. Effects of transplanted myoblasts transplanted with human growth hormone gene on improvement of ventricular function of rats. *Chin Med J*. 2008; 121: 347–54.
22. Yu G, Dai H, Chen J, et al. Gene delivery of indoleamine 2,3-dioxygenase prolongs cardiac allograft survival by shaping the types of T-cell responses. *J Gene Med*. 2008; 10: 754–61.
23. Gao F, He T, Wang H, et al. A promising strategy for the treatment of ischemic heart disease: mesenchymal stem cell-mediated vascular endothelial growth factor gene
transfer in rats. Can J Cardiol. 2007; 23: 891–8.

24. Jo J, Nagaya N, Miyahara Y, et al. Transplantation of genetically engineered mesenchymal stem cells improves cardiac function in rats with myocardial infarction: benefit of a novel nonviral vector, cationized dextran. Tissue Eng. 2007; 13: 313–22.

25. Mazighi M, Tchêché D, Gouëffic Y, et al. Percutaneous transplantation of genetically-modified autologous fibroblasts in the rabbit femoral artery: a novel approach for cardiovascular gene therapy. J Vasc Surg. 2006; 44: 1067–75.

26. Spiegelstein D, Kim C, Zhang Y, et al. Combined transmyocardial revascularization and cell-based angiogenic gene therapy increases transplanted cell survival. Am J Physiol Heart Circ Physiol. 2007; 293: H3311–6.

27. Bao C, Guo J, Lin G, et al. TNFR gene-modified mesenchymal stem cells attenuate inflammation and cardiac dysfunction following MI. Scand Cardiovasc J. 2008; 42: 56–62.

28. Dai Y, Ashraf M, Zuo S, et al. Mobilized bone marrow progenitor cells serve as donors of cytoprotective genes for cardiac repair. J Mol Cell Cardiol. 2008; 44: 607–17.

29. Liu XH, Bai CG, Xu ZY, et al. Therapeutic potential of angiogenin modified mesenchymal stem cells: angiogenin improves mesenchymal stem cells survival under hypoxia and enhances vasculogenesis in myocardial infarction. Microvasc Res. 2008; 76: 23–30.

30. Sun L, Cui M, Wang Z, et al. Mesenchymal stem cells modified with angiopoietin-1 improve remodeling in a rat model of acute myocardial infarction. Biochem Biophys Res Commun. 2007; 357: 779–84.

31. Zhou YL, Zhang HF, Li XL, et al. Increased stromal-cell-derived factor 1 enhances the homing of bone marrow derived mesenchymal stem cells in dilated cardiomyopathy in rats. Chin Med J. 2010; 123: 3282–7. The Authors

32. Wen Z, Zheng S, Zhou C, et al. Repair mechanisms of bone marrow mesenchymal stem cells in myocardial infarction. J Cell Mol Med. 2011; 15: 1032–43.

33. Klopsch C, Furiani D, Gabel R, et al. Intracardiac injection of erythropoietin induces stem cell recruitment and improves cardiac functions in a rat myocardial infarction model. J Cell Mol Med. 2009; 13: 664–79.

34. Frangogiannis NG, Entman ML. Chemokines in myocardial ischemia. Trends Cardiovasc Med. 2005; 15: 163–9.

35. Lienh EA, Tuchsheeaeer N, Kanzler I, et al. Double-edged role of the CXCL12-CXCR4 axis in experimental myocardial infarction. J Am Coll Cardiol. 2011; 58: 2415–23.

36. Lee BC, Lee TH, Avraham S, et al. Involvement of the chemokine receptor CXCR4 and its ligand stromal cell-derived factor 1alpha in breast cancer cell migration through human brain microvascular endothelial cells. Mol Cancer Res. 2004; 2: 327–38.

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