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Local injection of stem cell factor (SCF) improves myocardial homing of systemically delivered c-kit + bone marrow-derived stem cells

Matthias Lutz[1†], Mark Rosenberg[1†], Fabian Kiessling[2], Volker Eckstein[3], Thomas Heger[1], Jutta Krebs[1], Anthony D. Ho[3], Hugo A. Katus[1] and Norbert Frey[1*]

[1]*Department of Cardiology, Internal Medicine III, University of Heidelberg, INF 410, D-69120 Heidelberg, Germany;*
[2]*Department of Medical Physics in Radiology, German Cancer Research Center, INF 280, D-69120 Heidelberg, Germany and*
[3]*Department of Hematology, Internal Medicine V, University of Heidelberg, INF 410, D-69120 Heidelberg, Germany*

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Aims Recent studies have shown that stem cell therapy may alleviate the detrimental effects of myocardial infarction. Yet, most of these reports observed only modest effects on cardiac function, suggesting that there still is need for improvement before widespread clinical use. One potential approach would be to increase migration of stem cells to the heart. We therefore tested whether local administration of stem cell factor (SCF) improves myocardial homing of intravenously infused lin-/c-kit+ stem cells after myocardial infarction.
Methods and results Myocardial infarction was induced in mice via ligation of the left anterior descending artery and 2.5 μg of SCF were injected into the peri-infarct zone. Sham-operated mice and animals with intramyocardial injection of phosphate-buffered saline (PBS) served as controls. Twenty-four hours after myocardial infarction, lin-/c-kit+ stem cells were separated from murine bone marrow by magnetic cell sorting, labelled with the green fluorescent cell tracker CFDA or ^{111}Indium, and subsequently 750 000 labelled cells were systemically infused via the tail vein. Another 24 or 72 h later, respectively (i.e. 48 and 96 h after myocardial infarction), hearts were removed and analysed for myocardial homing of stem cells. Green fluorescent stem cells were exclusively detected in the peri-infarct zone of animals having prior SCF treatment. Radioactive measurements revealed that an intramyocardial SCF injection significantly amplified myocardial homing of lin-/c-kit+ stem cells compared to animals with PBS injections (3.58 ± 0.53 vs. 2.28 ± 0.23 cpm/mg/10^6cpm, +60%, $P < 0.05$) and sham-operated mice without myocardial infarction (3.58 ± 0.53 vs. 1.95 ± 0.22 cpm/mg/10^6cpm, +85%, $P < 0.01$). Similar results were obtained 72 h after stem cell injection.
Conclusion We demonstrate that intramyocardial administration of SCF sustainably directs more lin-/c-kit+ stem cells to the heart. Future studies will have to show whether higher levels of myocardial SCF (i.e. by virus-mediated gene transfer) can further improve homing of systemically delivered c-kit+ stem cells and thus favourably influence cardiac remodelling following myocardial infarction.

1. Introduction

The concept of the heart as a terminally differentiated organ unable to replace its myocytes has long been held in cardiovascular research. Accordingly, it has generally been accepted that the postmitotic heart can only sustain an increased workload by hypertrophy of existing myocytes rather than by addition of newly formed cardiomyocytes.[1] More recently, however, this dogma has been challenged by the identification of a significant number of Y chromosome positive endothelial and myocardial cells in female allografts after gender-mismatched heart transplantation.[2,3] Similarly, patients with a male-to-female bone marrow transplantation display myocardial repopulation of Y chromosome positive cardiomyocytes.[4] Finally, Jackson *et al.*[5] were able to show that bone marrow transplanted mice subjected to myocardial infarction reveal cardiomyocytes of exogenous origin, consistent with the hypothesis that bone marrow cells can give rise to cardiomyocytes and, at least in principle, could regenerate the myocardium.

Numerous studies have thus subsequently examined a potential therapeutic effect of bone marrow-derived cells

† These authors contributed equally to this study

* Corresponding author. Tel: +49 6221 561505; fax: +49 6221 568647.
E-mail address: norbert.frey@med.uni-heidelberg.de, freynorbert@hotmail.com

on myocardial function and regeneration after experimental myocardial infarction (reviewed by Murry *et al.*[6]). Whereas some of these studies provided evidence for extensive myocardial regeneration after cellular cardiomyoplasty,[7] others found no stem cells that had actually transdifferentiated into cardiomyocytes.[8,9] Yet, regardless of the variable effects on cardiac regeneration, virtually all of these studies found a significant improvement of cardiac contractile function after cellular therapy. Therefore, it is now generally accepted that stem cell therapy can favourably affect cardiac remodelling after myocardial infarction, but the scientific basis of this effect still remains unclear. Two recent studies hypothesized that stem cells may exert their beneficial influence on cardiac repair by supporting neoangiogenesis rather than by transdifferentiation into cardiomyocytes.[10,11] Several pathways and cytokines have been implicated in potentially mediating such an effect, including PI3K/akt,[11] thymosin ß4,[12] hepatocyte growth factor[13] as well as stem cell factor (SCF)/c-kit.[10] The latter pathway is of particular interest, since most of the bone marrow-derived stem cells so far utilized for cardiac repair are c-kit positive. C-kit is a protooncogene that maps to the white spotting locus (w) and encodes for a receptor tyrosine kinase. C-kit is highly expressed in adult haematopoietic stem cells and binds to its endogenous ligand SCF, which maps to the steel (Sl) locus.[14] Germline mutations in the Sl or W locus revealed that SCF/c-kit signalling is critical for proliferation, differentiation, and migration of immature cells during spermatogenesis and melanocytogenesis.[15,16] Furthermore, it has been shown that c-kit enriched haematopoietic stem cells have the potential to alleviate post-ischaemic injury in the myocardium.[7,9,10]

Based on these studies, we were asking if increased levels of SCF in the myocardium might direct more bone marrow-derived c-kit positive stem cells to the infarcted heart. To test this hypothesis, we induced myocardial infarctions in mice and administered recombinant SCF into the peri-infarcted area. Next, c-kit positive stem cells that were either marked by a green fluorescent cell tracker or by radioactive labelling were systemically applied by tail vein puncture. We show here that after pre-treatment with SCF significantly more lineage negative (lin-)/c-kit+ cells can be detected in infarcted mouse hearts, suggesting that the novel approach of local SCF delivery improves myocardial homing of bone marrow-derived stem cells.

2. Methods

2.1 Isolation, purification, and labelling of lin-/c-kit+ stem cells

For extraction of lin-/c-kit+ stem cells, the magnetic-activated cell sorting (MACS)-kit from Miltenyi Biotech was used and processed according to the manufacturer's protocol. Briefly, tibias and femurs of C57BL/6 mice were collected and flushed with phosphate-buffered solution (PBS) containing 2% FCS. To remove cell clumps, crude bone marrow was filtered through a 30 μm nylon mesh (Miltenyi Biotech). For the purpose of depleting mature haematopoietic cells such as T cells, B cells, monocytes/macrophages, granulocytes, erythrocytes as well as their committed precursors, cells were incubated with a 'cocktail' of biotinylated antibodies against a panel of 'lineage' (lin) antigens, including CD5, CD45R (B220), CD11b, Anti-Gr-1 (Ly-6G/C), 7-4, and Ter-119 (Lineage Cell Depletion Kit, Miltenyi Biotech). After addition of anti-biotin microbeads, lin-positive cells were separated using a magnetic column. Enrichment of the lineage negative cells for a subpool of cells expressing the stem cell marker c-kit/CD117 was performed in the same way by application of CD117 microbeads (Miltenyi Biotech).

The purity of the separated cells was assessed by fluorescence-activated cell sorting (FACS) using a phycoerythrin labelled antibody against CD117 (Pharmingen). Integrity and viability of purified lin-/c-kit+ cells was confirmed by propidium iodide (PI) (Sigma) staining. In order to be able to detect systemically delivered stem cells after potential engraftment in the heart *in vivo*, lin-/c-kit+ cells were labelled with the green fluorescent 'cell tracker' carboxyfluorescein diacetate (CFDA) succinimidyl ester (TefLabs). Briefly, lin-/c-kit+ stem cells were incubated for 30 min at 37°C with 5 μg/mL CFDA per 10^6 cells. The staining process was concluded with two washing steps to assure clearance of any unbound CFDA. For intravenous infusion, CFDA-labelled stem cells were collected in serum-free PBS and immediately injected. Radioactive labelling of lin-/c-kit+ cells was performed by incubation of stem cells with 1 μCi ^{111}Indium-oxine (^{111}In) (Tyco Healthcare, Germany) per 10^6 cells for 15 min at room temperature. Subsequently, two washing steps removed any unbound radioactivity. Radioactively labelled lin-/c-kit+ stem cells were again collected in serum-free PBS and injected intravenously via the tail-vein.

2.2 Induction of myocardial infarction and application of lin-/c-kit+ stem cells

To induce a myocardial infarction, female C57BL/6 mice at an age of 8–12 weeks were intubated and ventilated with 2% isoflurane (Minivent, HSE). After a left-sided thoracotomy, the left anterior descending (LAD) coronary artery was occluded by a permanent ligation (8–0 suture, Ethicon). Myocardial ischaemia was affirmed by pale decolourisation of the depending myocardium. This operation technique was adapted from the first description by Michael *et al.*[17] Infarct size was determined in four animals 7 days after the procedure. Scar volume was digitally (Image J) calculated by planimetric analysis of representative haematoxylin-eosin (HE) stained adjacent sections of the infarcted heart. Following this procedure, a total of 2.5 μg recombinant mouse stem cell factor (rSCF; Sigma-Aldrich) dissolved in 5 μL PBS was injected into 3–5 sites of the peri-infarct zone using a micro syringe (Hamilton) and a 31G canula. Sham injections were performed with identical volumes of PBS without rSCF. After closure of the thorax, anaesthesia was stopped and the mice were weaned from the respirator. Postoperative analgesia was ensured with intraperitoneal injections of Buprenorphin (Essex Pharma, Germany). Twenty-four hours after the surgical procedure 750.000 labelled (CFDA or ^{111}Indium) lin-/c-kit+ cells in 200 μL PBS were intravenously administered via the lateral tail vein. To verify complete intravenous application, we used a 27G canula connected to a short polyethylen-catheter. Reflux of blood indicated a secure intravenous position of the canula and allowed complete infusion of the stem cells. After additional 24 or 72 h, respectively, the animals were sacrificed by a lethal dose of thiopental (ALTANA Pharma, Germany) and the organs were harvested. For quantification of radioactivity, hearts were weighed and subsequently measured in a gamma counter (Perkin Elmer/Packard). Collected data were normalized to organ weight and applied activity (cpm per mg organ weight per 10^6 cpm injected activity—cpm/mg/10^6 cpm infused). Biometric data of the animals allocated to three different treatment groups (no myocardial infarction, $n = 15$; myocardial infarction with PBS-injection, $n = 16$; myocardial infarction with SCF-injection, $n = 16$) revealed no significant difference (body weight in g: 20.7 ± 0.42; 21.2 ± 0.61; and 19.8 ± 0.52, $P =$ n.s.; heart weight in mg: 104.9 ± 1.43; 117.8 ± 5.2; 119.8 ± 5.32 respectively, $P =$ n.s.).

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.3 Imunostaining

Frozen tissue-blocks were embedded in TissueTec OCT (Sakura) and cut into 7 μm sections, fixed in acetone, and blocked with 2.5% bovine serum albumin (BSA) in PBS for 1 h. As primary antibodies, we used anti-c-kit (C19, Santa Cruz Biotechnology) (1:200), anti-SCF (R&D) (1:200), and anti-sarcomeric alpha-actinin (Sigma) (1:1000), respectively. Antibodies were diluted in blocking solution and incubated for 1 h at room temperature. As secondary antibodies, we utilized Cy3 antibodies against mouse-, rabbit- and goat-IgG (Dianova) at a dilution of 1:400. The incubation time was 30 min. Thereafter, slides were washed in PBS, covered with plastic plates, and fixed with mounting medium containing diamidino-2-phenylindol (DAPI) (Vectashield). The sections were examined using a Nikon 80i fluorescence microscope.

2.4 Transthoracic echocardiography

Heart function was assessed 2 weeks after stem cell application by echocardiography in anaesthetized mice (2.5% Avertin at 15 μL/g body weight), using a Hewlett Packard Sonos Ultrasound system. The examiner was blinded to the treatment of the mice and obtained three independent M-mode measurements per animal. In the short-axis view, left ventricular end-systolic (LVESD) and left ventricular end-diastolic (LVEDD) chamber diameters and thickness of the interventricular septum and the posterior wall were determined. In order to compare myocardial infarction, left ventricular fractional shortening ($FS = [(LVEDD - LVESD)/LVEDD] \times 100$) was calculated.

2.5 Statistical analysis

Data are presented as mean ± SEM. The multigroup analysis of the different infarct and sham groups were performed using ANOVA and the Neuman–Keuls multiple comparisons post test. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Magnetic cell sorting yields highly purified lin-/c-kit+ stem cells

Lin-/c-kit+ bone marrow cells were isolated from male C57BL/6 mice by magnetic cell sorting, typically resulting in a yield of $1.1–1.4 \times 10^8$ bone marrow cells per dissected mouse. After the separation process, approximately $0.9–1.2 \times 10^6$ lin-/c-kit+ stem cells were obtained, corresponding to ~1% of the used whole bone marrow cells. FACS analyses were performed to check for purity and viability of the separated stem cells (*Figure 1 A–C*). *Figure 1 A* shows that a fraction of 1.7% of whole bone marrow cells stains positive for c-kit. Magnetic cell sorting yielded a highly purified cell population with more than 90% of the cells positive for c-kit (*Figure 1 B*). In addition, more than 95% of the cells displayed no PI labelling, thereby confirming their viability (*Figure 1 C*).

3.2 Myocardial infarction and stem cell transplantation

The study design is outlined in *Figure 2*. Myocardial infarction was induced under general anaesthesia by direct ligation of the left descending artery (LAD). Determination of the infarct size in a subgroup of four animals revealed scars with a mean volume of $0.12 \pm 0.01\ cm^3$. This corresponded to approximately 25 ± 3.4% of the left ventricular mass (*Figure 3 A*), similar to previous reports applying this procedure.[17] Shortly after ligation of the LAD, animals were randomly assigned to an injection with 2.5 μg recombinant mouse SCF into the peri-infarct zone (total of 5 μL at 4–5 sites). Control animals received PBS injections. Twenty-four hours later, 750.000 lin-/c-kit+ cells were labelled with the green fluorescence marker CFDA and subsequently injected into the tail vein by an investigator unaware of the nature of the preceding procedure (SCF vs. PBS). At this timepoint, intramyocardial SCF deposits were still readily detectable by immunostaining (*Figure 3 B*).

3.3 Lin-/c-kit+ stem cells are detected in stem cell factor-treated mouse hearts

To detect intravenously administered lin-/c-kit+ cells in the myocardium, we analysed tissue sections by immunostaining and fluorescence microscopy. Systemically applied stem cells could be differentiated from endogenous cardiac cells using the cell tracker CFDA. One day after application, fluorescent cells were detected in the border zone of the infarction. Interestingly, CFDA-positive cells were typically found in small clusters (*Figure 4*). Of note, we were only able to detect these cell clusters in hearts with prior SCF treatment. In contrast, in control animals with PBS-injection, no CFDA-positive cells could be identified, suggesting that an increase in local SCF abundance facilitated homing of systemically administered c-kit+ stem cells. In addition, the vast majority of green-fluorescent cells also stained positive for c-kit (*Figure 5 A–C*), further supporting the notion that exogenous c-kit+ stem had homed to SCF-treated mouse hearts. On the other hand, we could not detect any cells with c-kit expression without a concomitant CFDA signal, implying a lack of endogenous c-kit+ cells in the mouse heart 24 h post myocardial infarction. In contrast, a recent publication by Fazel *et al.*[10] reported that 7 days after an experimentally induced myocardial infarction, an increased number of endogenous c-kit+ stem cells from the bone marrow can be found in the heart. This apparent discrepancy might be explained by the earlier time point we used to track transplanted lin-/c-kit+ bone marrow stem cells. Counterstaining of representative tissue-slides of hearts excised 24 and 72 h after stem cell injection with an antibody against alpha-actinin revealed no simultaneous signals for green fluorescent stem cells and the sarcomeric protein alpha-actinin (*Figure 5 D–F*). Therefore, no evidence was found for transdifferentiation of the engrafted cells into cardiomyocytes, at least up to 72 h post transplantation.

3.4 Quantification of radioactively labelled lin-/c-kit+ stem cells reveals a significant improvement of myocardial homing in response to local stem cell factor treatment

As described earlier, engrafted c-kit+ stem cells of exogenous origin could be detected in infarcted mouse hearts post local SCF treatment. In order to quantify the improved stem cell homing to the heart, we used radioactive labelling of the lin-/c-kit+ stem cells. For this purpose, c-kit+ stem cells were labelled with the gamma emitter ^{111}Indium and again systemically administered via tail vein puncture. On the following day, animals were sacrificed and the hearts were removed. Radioactivity of the hearts was measured in a gamma counter. Mice that were treated with intramyocardial injection of SCF ($n = 9$) after induction of a

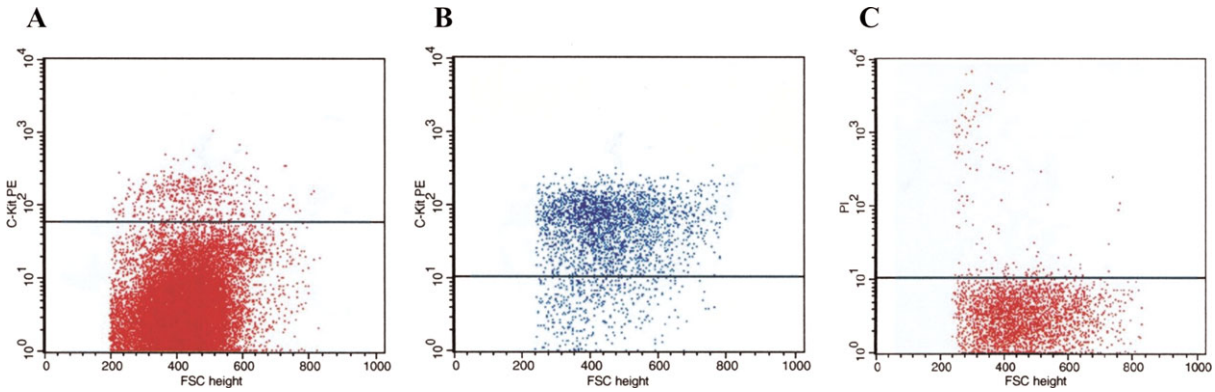

Figure 1 Fluorescence-activated cell sorting (FACS) analysis for characterization of lin-/c-kit positive stem cells: (*A*) FACS analysis of whole unseparated murine bone marrow cells. Cells were labelled with a c-Kit-PE antibody. Quantitative analysis revealed that 1.7% of whole bone marrow cells stained positive for c-kit. (*B*) After the separation process, 90% of the purified stem cells were c-kit positive; (*C*) in order to test for viability of the separated stem cells, propidium iodide (PI) staining was performed. Up to 95% of the screened stem cells lack PI -incorporation, thereby confirming viability in the vast majority of purified lin-/c-kit+ stem cells.

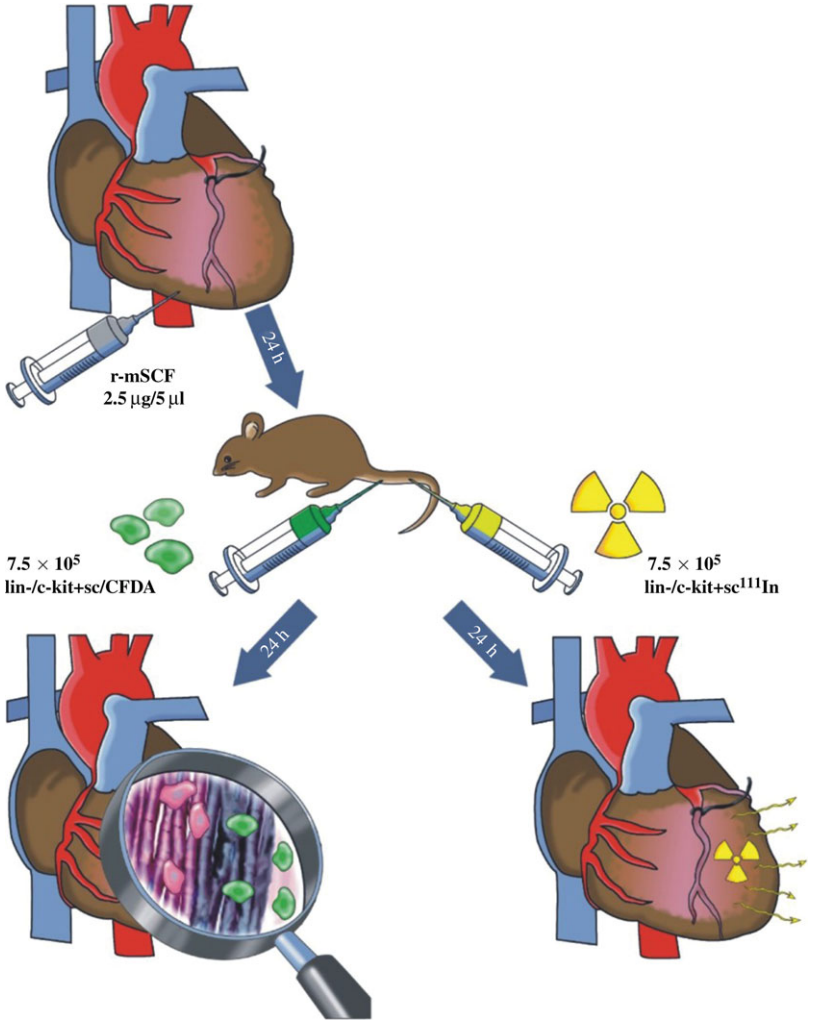

Figure 2 Cartoon of the study design. Myocardial infarction was induced in mice via ligation of the left anterior descending artery and recombinant mouse stem cell factor (r-mSCF) was injected into the peri-infarct zone. Lin-/c-kit+ stem cells (lin-/c-kit+sc) were either labelled with the green fluorescent cell tracker carboxyfluorescein diacetate (CFDA) or ^{111}Indium (^{111}In). Twenty-four hours later, 750.000 labelled cells were systemically infused via the tail vein. After another 24 h, the animals were sacrificed and the organs were harvested. Potential engraftment of CFDA positive cell in the myocardium was analysed by immunostaining of tissue sections. In order to quantify myocardial stem cell homing, radioactivity of the hearts was measured in a gamma counter.

myocardial infarction revealed a significant 60% increase of emitted radiation compared to controls with a sham-injection of PBS ($n = 10$) (3.58 ± 0.53 vs. 2.28 ± 0.23 cpm/mg/10^6cpm infused, $P < 0.05$; *Figure 6 A*). This effect remained statistically significant irrespective of the normalization to applied radioactive activity and/or organ weight (data not shown). When compared to hearts that neither had a myocardial infarction nor a cytokine injection

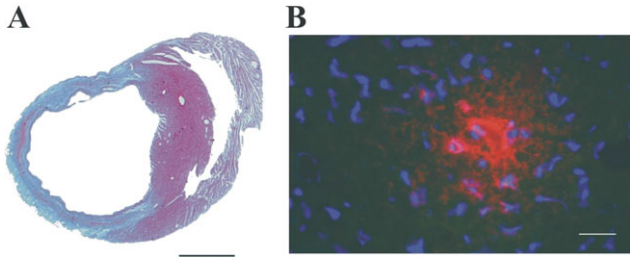

Figure 3 (*A*) Myocardial infarction and infarct size: HE staining of a representative slide through the infarcted heart 1 week after permanent ligation of the left anterior descending. At this time, HE-staining and left ventricular thinning clearly delineates the infarct zone from the spared myocardium. Scale bar: 1 mm. (*B*) Intramyocardial deposition of recombinant mouse stem cell factor (SCF): tissue section showing the patchy distribution of recombinant SCF in the myocardium 24 h after direct intramyocardial injection. Recombinant SCF is detected by a polyclonal SCF-antibody. Scalebar: 50 μm.

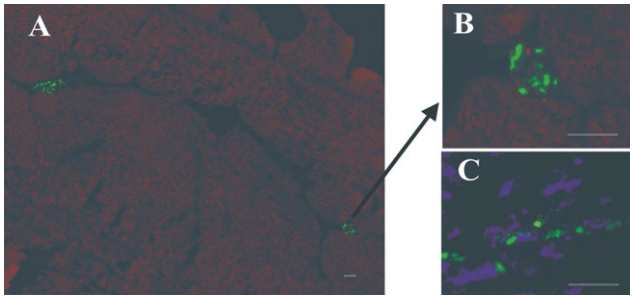

Figure 4 Detection of intramyocardial carboxyfluorescein diacetate (CFDA)-positive cells in infarcted hearts with prior stem cell factor treatment. (*A*) Clusters of CFDA-positive cells were detected in the peri-infarct zone. (*B*) Magnified view of a representative CFDA positive cell cluster. (*C*) Overlay of CFDA positive cells with DAPI.

($n = 9$), the combination of myocardial infarction and SCF treatment boosted the difference to 85% (3.58 ± 0.53 vs. 1.95 ± 0.22 cpm/mg/10^6cpm infused, $P < 0.01$). Myocardial infarction alone without SCF application resulted in a moderate trend towards increased stem cell homing, but failed to reach statistic significance (2.28 ± 0.23 vs. 1.95 ± 0.22 cpm/mg/10^6cpm infused, $P > 0.05$) (*Figure 6 A*). To differentiate myocardial engraftment of the injected lin-/c-kit+ stem cells from a transient phenomenon, a second point of time was chosen according to ^{111}Indiums half time. Seventy-two hours after stem cell injection animals of the three subgroups were sacrificed and the hearts were removed. Again, hearts of mice that were treated with an intramyocardial injection of SCF ($n = 7$) showed a significant increase in emitted radiation when compared to animals with myocardial infarction and intramyocardial sham injection ($n = 6$) or to control animals who neither had an infarction nor an intramyocardial injection ($n = 6$) (1.47 ± 0.08 vs. 1.18 ± 0.07 vs. 1.07 ± 0.11 cpm/mg/10^6cpm infused; $P < 0.05$). Myocardial infarction alone without cytokine application only led to a moderate trend towards improved stem cell homing, but again did not reach statistic significance (1.18 ± 0.07 vs. 1.07 ± 0.11 cpm/mg/10^6cpm infused; $P =$ n.s.).

4. Discussion

While it is still controversial if and to which extent stem cells can actually regenerate infarcted myocardium, several experimental as well as early clinical studies revealed an improved contractile function due to cell therapy, thereby confirming potentially favourable effects of this novel treatment modality.[18] However, in most of these reports, only modest effects on cardiac function were observed, suggesting that there is still a need for significant improvement of stem cell-based therapy before widespread clinical use. One potential approach would be to increase migration and homing of stem cells to the myocardium. We were thus asking if homing of bone marrow-derived stem cells could be enhanced by local delivery of cytokines that could direct these cells to the infarcted heart.

C-kit and its endogenous ligand SCF have been shown to be important for mobilization and migration of immature cells.[16,19,20] We thus hypothesized that local delivery of SCF would improve migration of lin-/c-kit+ stem cells to the infarcted heart. Here we demonstrate that direct injection of recombinant SCF into the peri-infarct zone indeed directed significantly more lin-/c-kit+ stem cells of exogenous origin to the infarcted heart compared to control mice without SCF injection. Quantification of myocardial homing was performed by radioactive labelling of haematopoietic stem cells with ^{111}Indium. In order to exclude a transient phenomenon, measurements were conducted at two different time points (24 and 72 h), revealing a sustained engraftment of systemically injected stem cells in the myocardium. Due to the rapid radioactive decay of ^{111}Indium, later time points could not be assessed.

To the best of our knowledge, this is the first report to show that c-kit positive stem cells applied through a peripheral vein can be attracted to the heart by local injection of a cytokine. What could be the mechanism of this finding? Homing of undifferentiated cells to a peripheral organ consists of a two-step process that begins with binding of stem cells to adhesive molecules in the vasculature followed by local chemotaxis to the site of engraftment. Myocardial accumulation of SCF could serve as an adhesive factor for c-kit positive cells by a direct receptor-ligand interaction in the microcirculation of the heart. Furthermore, a gradient for c-kit positive stem cells could be created by local injection of SCF, thereby potentially facilitating their chemotactic attraction. In line with this notion, we observed exogenous bone marrow-derived stem cells only in the infarct border zone close to the sites of SCF injection. Also, these cells were typically found in small clusters adjacent to small blood vessels, consistent with transmigration from the vasculature. As a result, we noted a significantly higher number of c-kit positive stem cells in the heart after cytokine treatment. A similar observation has been made by Sun *et al.*,[19] who assessed the potential of SCF to direct c-kit+ neural stem cells to injured brain tissue: recombinant SCF strongly induced migration of stem cells through activation of c-kit both *in vitro* and *in vivo*. Likewise, Kunisada *et al.*[16] reported a transgenic mouse model in which overexpression of SCF in keratinocytes led to extensive migration of c-kit positive melanocyte precursors to the skin. Taken together, these findings as well as our data suggest that SCF can act as a potent chemoattractant for c-kit positive cells *in vivo*.

Yet, other mechanisms may also contribute to the observed improvement of myocardial homing of lin-/c-kit+ cells upon SCF treatment. In this regard, it has been shown that the SCF/c-kit interaction promotes cell

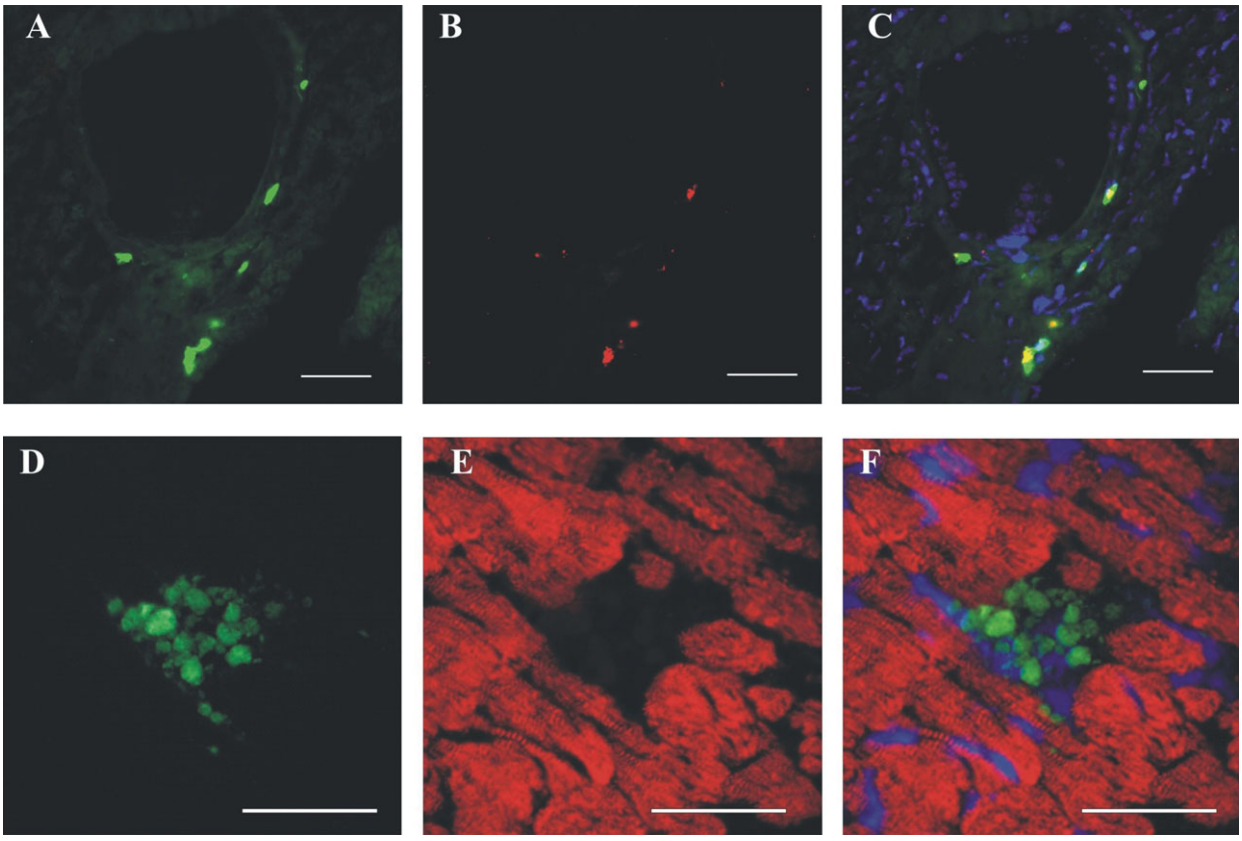

Figure 5 Co-staining of carboxyfluorescein diacetate (CFDA)-positive cells for c-kit and alpha-actinin reveals maintenance of an undifferentiated phenotype. (*A–C*) Green-fluorescent CFDA-labelled cells (A) also stain positive for c-kit (B) merged with nuclear DAPI staining, indicating that the cells kept their undifferentiated state (C). Note the typical perivascular clustering of systemically delivered exogenous c-kit positive cells. (*D–F*) Green-fluorescent CFDA-labelled cells (D) are negative for alpha-actinin (E) merged with DAPI staining, suggesting that lin-/c-kit+ stem cells did not transdifferentiate into cardiomyocytes(F). Scalebar: 50 μm.

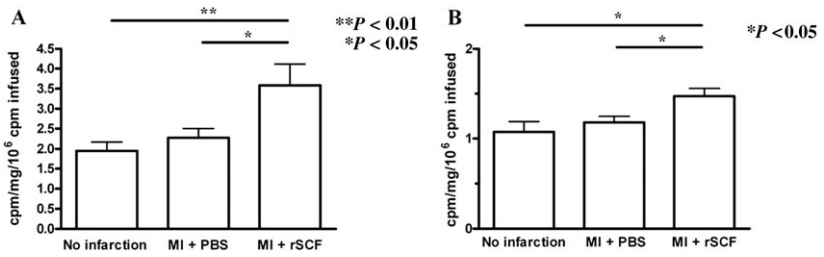

Figure 6 Stem cell factor (SCF) pre-treatment enhances myocardial homing of systemically delivered radioactively labelled stem cells. (*A*) Mice treated with an intramyocardial injection of recombinant stem cell factor (rSCF) ($n = 9$) after induction of myocardial infarction (MI) revealed a significant 60% increase of emitted radiation compared to controls with a sham-injection of PBS ($n = 10$) (3.58 ± 0.53 vs. 2.28 ± 0.23 cpm/mg/10^6cpm infused, $P < 0.05$) 24 h post infusion. When compared to hearts that neither had a myocardial infarction nor a cytokine injection ($n = 9$), the combination of myocardial infarction and SCF treatment increased the difference to 85% (3.58 ± 0.53 vs. 1.95 ± 0.22 cpm/mg/10^6 cpm infused, $P < 0.01$). Myocardial infarction alone without SCF application resulted in a moderate trend towards increased stem cell homing, but failed to reach statistic significance (2.28 ± 0.23 vs. 1.95 ± 0.22 cpm/mg/10^6cpm infused, $P > 0.05$). Data are presented as mean+SEM. (*B*) Seventy-two hours after stem cell application similar results were obtained. Hearts of mice that were treated with an intramyocardial injection of SCF ($n = 7$) still showed a significant increase in emitted radiation when compared to animals with myocardial infarction and intramyocardial sham injection ($n = 6$) or to control animals who neither had an infarction nor an intramyocardial injection ($n = 6$) (1.47 ± 0.08 vs. 1.18 ± 0.07 vs. 1.07 ± 0.11 cpm/mg/10^6cpm infused; $P < 0.05$). Myocardial infarction alone without cytokine application only led to a moderate trend towards improved stem cell homing, but failed to reach statistic significance (1.18 ± 0.07 vs. 1.07 ± 0.11 cpm/mg/10^6 cpm infused; $P =$ n.s.). Data are presented as mean+SEM.

survival by inhibition of apoptosis in multiple cell types, including haematopoietic progenitor cells. For example, activation of the receptor tyrosine kinase c-kit by SCF in neurons led to phosphorylation of the antiapoptotic kinase Akt via PI3K and thus conferred a neuroprotective effect.[21] Therefore, it is possible that our observation of an increased number of c-kit positive cells can at least in part be attributed to improved survival of these cells via activation of antiapoptotic pathways.

Regardless of the underlying mechanism, there is accumulating evidence that an increased number of c-kit positive cells improve healing and remodelling of the infarcted heart. First, Orlic *et al.*[7] demonstrated that direct transplantation of c-kit positive bone marrow cells into the border zone of infarcted mouse myocardium led to a significant improvement of contractile function. While Balsam *et al.*[9] in a very similar experiment could not confirm actual transdifferentiation of the transplanted cells into

cardiomyocytes, they nevertheless observed a significant increase in fractional shortening of cell-treated hearts. More recently, Fazel *et al*.[10] provided a potential explanation for these advantageous effects even in the absence of newly formed cardiomyocytes: mice with compound heterozygosity for two inactivating mutations of the c-kit receptor displayed a marked reduction in contractile function as well as survival post myocardial infarction compared to wild-type mice. Conversely, transplantation of wild-type, GFP-labelled bone marrow cells rescued the cardiomyopathic phenotype. Mechanistically, these authors could show that the beneficial effect of c-kit+ cells in the heart depends on their ability to create a pro-angiogenic milieu in the infarct border zone with increased expression of VEGF. Interestingly, neither transdifferentiation of c-kit+ bone marrow cells into cardiomyocytes was required for the therapeutic effect, nor the permanent engraftment of these cells in the infarcted myocardium, since very few GFP-positive cells could still be detected 28 days post transplantation.[10] In our experimental model, intramyocardial SCF application led to a patchy and localized distribution pattern. While this approach resulted in considerably higher stem cell numbers in the heart, only a small absolute amount (approximately 400 cells of the initially injected c-kit positive cells) homed to the heart. It was therefore not surprising that increased myocardial homing did not translate into a functional benefit in our experimental set-up, as revealed by echocardiography at 14 days (Supplementary material online, *Figure S1*).

What are the potential clinical implications of these findings? Several investigators already reported small-scale clinical trials in which (usually unfractionated) autologous bone marrow cells were infused into the coronary arteries of patients with a recent myocardial infarction. While some of these studies observed a modest improvement of the ejection fraction in the treatment group,[22,23] there is still no evidence to suggest that the transplanted cells transdifferentiated to cardiomyocytes. This has led to the hypothesis that bone marrow cells infused into the coronaries rather provide cytokines and growth factors which in turn favourably modulate the cardiac adaptation to myocardial infarction.[11,24] While it seems likely that several such factors will be identified, we believe that SCF could be such a molecule which facilitates homing of c-kit positive stem cells to the heart and thereby potentially mediates a therapeutic effect.

Conclusion

In summary, we provide the 'proof of concept' that enrichment of SCF in the infarcted heart results in significantly increased homing of systemically applied lin-/c-kit+ bone marrow-derived stem cells. Future experiments will have to show if higher levels of SCF homogenously distributed throughout the myocardium (i.e. by virus-mediated gene transfer or transgenesis) can further enhance migration of systemically delivered c-kit+ stem cells to the injured heart. Finally, it will be critical to test if the SCF-mediated enrichment of c-kit positive cells also translates into improved contractile function and thus a favourable effect on cardiac remodelling post myocardial infarction.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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