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Renin Inhibition Improves the Survival of Mesenchymal Stromal Cells in a Mouse Model of Myocardial Infarction

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Abstract

The aim of this study was to determine if renin inhibition is able to improve the survival of transplanted stem cells in a mouse model of myocardial infarction. Myocardial infarction was induced in FVB/NJ inbred mice ($n=23$). Bone marrow-derived mouse mesenchymal stromal cells (mMSCs, 3×10^5) expressing the reporter gene firefly luciferase were delivered intramyocardially ($n=12$) and monitored non-invasively by bioluminescence imaging. A group of these mice ($n=6$) received aliskiren (15 mg/kg/day) via an osmotic pump implanted subcutaneously. The survival of mMSCs was significantly increased in those animals that received aliskiren leading to a significant improvement in systolic function after myocardial infarction. Histological analysis revealed a

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significant reduction in inflammation and collagen deposition in those mice that received aliskiren compared to controls. Renin inhibition of the ischemic myocardium is able to modulate the microenvironment improving the survival and efficacy of transplanted mMSCs in a mouse model of myocardial infarction.

Keywords

Myocardial infarction; Mesenchymal stromal cells; Aliskiren; Renin-angiotensin system; Stem cell survival

Introduction

Stem cell-based intervention has recently emerged as an alternative approach for myocardial repair in coronary heart disease (CHD) [1–5]. Among the wide range of cell populations, mesenchymal stromal cells (MSCs) have been identified as one of the most attractive candidates for clinical application [6–8], particularly due to their immunomodulatory properties that could allow allogeneic transplantation [9]. Their beneficial effects have been exerted mainly through paracrine mechanisms rather than cellular differentiation, showing only modest improvements in left ventricular cardiac function [7, 10, 11].

However, previous studies have consistently shown that stem cells have poor survival after transplantation, what could constitute the major limitations in stem cell therapy, considerably diminishing its potential [5, 8, 6]. Due to this potential limitation, there has been significant interest in understanding the factors that determine the survival of transplanted stem cells. It has been suggested that stem cells transplanted to the myocardium can face a noxious environment that can limit their potential [12]. The ischemic myocardium is characterized by a hostile microenvironment in which interactive biological pathways, such as inflammation, fibrosis, and oxidative stress, play a pivotal role in cardiac function and tissue remodeling [13, 14]. This adverse myocardial environment can be deleterious to the survival and functionality of transplanted cells. Furthermore, the activation of the renin-angiotensin aldosterone system (RAAS), through pro-inflammatory and pro-fibrotic effects, has been singled as a key process in the left ventricular (LV) remodeling that occurs after myocardial infarction. In fact, blockades of the RAAS, either by angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin II receptor blockers (ARBs), have proven efficacious in modulating the noxious environment and limiting left ventricular remodeling after myocardial infarction. Thus, it is possible that modulation of the microenvironment can result in better survival of transplanted cells.

Because renin is the rate-limiting step in the conversion of angiotensinogen to angiotensin I (Ang I), it has been considered as the optimal target for RAAS inhibition, preventing the compensatory rise in plasma renin activity and other downstream components of this system, which occurs in the setting of ACE-I or ARBs therapy [15]. At the moment, aliskiren is considered the prototype of this class, with a great potential in the modulation of the myocardial microenvironment. However, while Westermann et al. [16] have recently shown that subpressor doses of aliskiren exert a beneficial effect on left ventricular systolic and diastolic function, as well as on cardiac hypertrophy and cardiomyocyte apoptosis in a

mouse model of myocardial infarction, in a clinical trial comparing aliskiren to placebo, no further attenuation of left ventricular remodeling was observed [17].

In this study, we sought to investigate whether modulation of the post-myocardial infarction environment, using a renin inhibitor strategy (aliskiren), would increase the survival of transplanted stem cells and ultimately improve the beneficial effect of stem cells on overall LV function in a mouse model of myocardial infarction.

Methods

Animals

Studies were performed on female FVB/NJ inbred mice (Jackson Laboratory, Bar Harbour, ME, USA) of 11 weeks of age, weighing 20–25 g. All animal experiments complied with the standards stated in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, MD, USA) and were approved by the Mayo Clinic College of Medicine Institutional Animal Care and Use Committee.

Experimental Design

Mice (11 weeks of age) underwent a surgical procedure for the induction of myocardial infarction (I) by left anterior descending coronary artery (LAD) ligation and were randomly divided into four groups: mice+PBS (I, $n=6$), mice+aliskiren (IA, $n=5$), mice+mesenchymal stromal cells, MSCs (IM, $n=6$), and mice+MSCs and aliskiren (IMA, $n=6$). Systolic blood pressure (SBP) was assessed non-invasively using the tail-cuff method at 10 weeks of age and 10 days post-surgery. Transplanted MSCs were monitored non-invasively by using bioluminescence imaging at 1, 2, 3, and 7 days post-surgery, while left ventricular (LV) systolic function was evaluated 2 days before and then 10 days after induction of myocardial infarction using a high-resolution ultrasound system. At 11 days post-surgery, mice were euthanized by carbon dioxide (CO₂) inhalation and tissue collected for ex vivo analysis.

In Vitro Studies

Isolation and Phenotypic Characterization of mMSCs—Allogeneic mMSCs were isolated from the bone marrow (BM) of FVB-Tg(CAG-luc,-GFP)L2G85Chco/J transgenic mice (Jackson Laboratory), using traditional plastic adherence isolation [18]. Briefly, 3-week-old animals were euthanized with carbon dioxide (CO₂), and BM aseptically collected by mincing femurs and tibias in cold (4 °C) Iscove's Modified Dulbecco's Medium (IMDM, STEMCELL Technologies, Vancouver, Canada) with 2 % fetal bovine serum (FBS). The mononuclear cell suspension was then filtered through a 70-µm cell strainer (BD Biosciences, San Jose, CA, USA), centrifuged at 500g for 5 min at 4 °C, and cultured in Minimum Essential Medium-alpha (αMEM, Gibco® Life Technologies, Grand Island, NY, USA) supplemented with 10 % FBS and 1 ng/ml basic fibroblast growth factor (bFGF, Sigma-Aldrich, St. Louis, MO, USA). Three days later, non-adherent cells were removed and adherent cells cultured for three more weeks. Medium was changed every 2–3 days until they reached 80–90 % confluence.

At passage 4, cellular phenotype was examined in vitro by fluorescence-activated cell sorting (FACS), using antibodies against CD45, CD29, CD90, CD44, and CD73 and the corresponding isotype control antibodies (all from Biologend, San Diego, CA, USA). The cells were positive for CD29, CD90, CD44, and CD73, but negative for the pan-hematopoietic cell marker CD45 (Fig. 1), as previously described [18].

For in vitro experiments, cells were first challenged with either low serum (1 % FBS) or TNF alpha (10–50 ng/ml) and then treated with aliskiren (50 μ M). The concentration of aliskiren was selected after performing a dose response study (0–100 μ M).

Cytotoxicity Assay—Cytotoxicity was measured with the lactate dehydrogenase (LDH) release assay (CytoTox 96 Nonradioactive assay, Promega, Madison, WI, USA) using the manufacturer's instructions, as previously described [19]. Total LDH released into medium and total cellular LDH were calculated, and results were expressed as percentage of total cellular LDH released into medium.

Assessment of Oxidative Stress—The production of the endogenous oxidative stress by-product hydrogen peroxide (H_2O_2) was assessed using the conversion of 2',7'-dichlorodihydrofluorescein diacetate (DCHFDA, Molecular probes, Eugene, OR, USA), as previously described [19]. Fluorescence was corrected for background signal, normalized for protein content, and expressed as relative fluorescent units (RFU) per microgram of protein.

Western Blotting—Protein expression in cell culture was evaluated by Western blotting following standard protocols [19], with β -actin (Cell Signaling Technology, Inc., Danvers, MA, USA) as the loading control. NAD(P)H oxidase p67^{phox} (EMD Millipore, Billerica, MA, USA) and Xanthine dehydrogenase (XDH) (Epitomics, Burlingame, CA, USA) were used to assess the activation of the oxidative stress pathway.

In Vivo Studies

Myocardial Infarction, Cell Delivery, and Osmotic Pump Implantation—Mice were anesthetized with isoflurane (4 %) and the left side of their chest shaved. Following endotracheal intubation, animals were placed supine on the surgical table over a heating pad and mechanically ventilated. Isoflurane (1.8 %) was used to maintain general anesthesia. Using sterile techniques, a left thoracotomy was performed in the intercostal space between the 4th and the 5th rib and the LV anterolateral wall exposed to visualize the LAD. A 9–0 Ethilon suture was inserted into the myocardium enclosing the LAD just 1.5 mm distal to the left auricle and closed using a triple surgeon's knot. Ischemia was confirmed by the appearance of pallor over the apical LV myocardium, along with hypokinesis/akinesis. Two injections of 15 μ l each, giving a total of 3×10^5 mMSCs, were administered transeptically through a 31-gauge needle into the border of the ischemic territory, 10 min after the LAD ligation (IM and IMA groups).

In order to deliver the renin inhibitor drug, aliskiren (15 mg/kg body weight/day, Novartis, Switzerland), a micro-osmotic pump (model 1004, Alzet, Cupertino, CA, USA), was implanted subcutaneously (IA and IMA groups).

Surgical closure was performed in three layers (intercostals, muscular, and cutaneous) using absorbable 6–0 silk sutures. Finally, residual air in the thoracic cavity was evacuated through a 25-gauge plastic cannula and mice extubated. Animals were supported on a thermal pad with supplemental oxygen during recovery and subcutaneous buprenorphine for analgesia (every 12 h during 3 days).

Blood Pressure Measurements—After a 1-week acclimatization period, systolic blood pressure was assessed in conscious mice by the tail-cuff method using the XBP1000 non-invasive blood pressure system (CODA system, Kent Scientific, Torrington, CT, USA). Fifteen measurements were obtained and averaged for each individual animal. The mean values of all analyses were used for comparison.

In Vivo Bioluminescence Imaging—Mice that received mMSCs (IM and IMA groups) underwent in vivo bioluminescent imaging (BLI) at 1, 2, 3, and 7 days post-surgery to detect Fluc signal (transgenic cells). This procedure was performed under isoflurane (1.5 %) anesthesia with the cooled charge-coupled device camera (Xenogen IVIS-200 optical in vivo imaging system). After intraperitoneal injection of the reporter substrate D-luciferin (50 mg/kg body weight), animals were imaged for 25 min using 5-min high-sensitivity acquisition scans. Bioluminescence was corrected for the baseline signal and was quantified as total radiance (photons/s/cm²/sr) over the area of the heart, using a region of interest that was kept at constant size for all scans.

Transthoracic Echocardiography—Two-dimensional and M-mode echocardiography acquisitions were performed at baseline (2 days before surgery) and 10 days after the induction of myocardial infarction with a dedicated small animal high-resolution ultrasound system (Hi-Res US, VeVo 770, VisualSonics, Inc., Toronto, ON, Canada), equipped with a 30-MHz mechanical transducer. Animals were anesthetized with 1.5 % of isoflurane during the whole procedure (10 min) and placed on a warming platform (set to 38 °C) in a supine position. The animal's heart rate, ECG signal, and respiration rate were recorded, and the body temperature monitored. The chest was shaved, and Aquasonic 100 gel (Parker Laboratories, Fairfield, NJ, USA) was applied to the thorax surface to optimize visibility of the cardiac chambers. Parasternal long-axis and parasternal short-axis two-dimensional views were acquired. For the assessment of LV systolic function, the M-mode of the parasternal long- and short-axis view at both the papillary muscle and apex level was used. In these views, the parameters calculated were as follows: LV end-systolic and end-diastolic

volumes ($ESV = \left(\frac{7.0}{2.4 + LVID_s} \right) \times LVID_s^3$ and $ESD = \left(\frac{7.0}{2.4 + LVID_d} \right) \times LVID_d^3$, where LVIDs and LVIDd represent the LV internal diameter at end-systole and end-diastole, respectively), as well as left ventricular ejection fraction (LVEF), expressed as the fraction of

blood volume ejected after each heart beat ($LVEF = \frac{(EDV - ESV)}{EDV} \times 100$).

Ex Vivo Studies

Immunohistochemical Staining—Heart tissues were fixed in 10 % neutral buffered formalin, dehydrated and embedded in paraffin, and histologic sections (5-μm thick) were

prepared. Representative sections were stained with hematoxylin and eosin for whole myocardial infarction size and structure and Picrosirius Red for fibrosis. Images were acquired with the NanoZoomer Digital Pathology system (Hamamatsu Photonics, Hamamatsu, Japan). Quantitative analysis of fibrotic area was performed in whole heart sections using MetaMorph microscopy automation and image analysis software (Molecular Devices, LLC, Sunnyvale, CA, USA). The thickness of the infarcted area was measured in six different points (when possible) along the length of the ischemic region and then averaged.

Additional unstained slides were prepared for immunohistochemical staining including NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells, Abcam, Cambridge, MA, USA, 1:200), TGFbeta (Abcam, 1:100), and vWf (von Willebrand factor, Abcam, 1:5,000). Antigen retrieval was performed by heat treatment in citrate buffer (pH 6.0) for 20 min, using a microwave for NFkB stain, or a commercial vegetable steamer for TGFbeta stain and by enzymatic treatment with Proteinase K (Dako North America, Inc., Carpinteria, CA, USA, 10 min at 37 °C) for vWf stain. Color was developed using DAB (Dako North America, Inc.) followed by hematoxylin counterstain. Coverslips were mounted with Permount mounting media. Images were acquired using a Nikon Eclipse 50i clinical microscope (Nikon Instruments Inc., Melville, NY, USA) and Spot Advanced modular image software (Spot™ Imaging Solutions, Sterling Heights, MI, USA). Quantitative analysis of positive-stained area was performed using MetaMorph software and averaged from four random fields of view (×20 magnification) for each of the border and infarcted regions per section, per animal.

Statistical Analysis

Statistical comparisons between groups were performed using non-parametric Kruskal–Wallis test (with Dunn’s multiple comparison test) or non-parametric Mann–Whitney test, as appropriate. Results are expressed as mean±standard error (SEM) of multiple experiments. Statistical significance was established at two-tailed $P<0.05$. All analyses were conducted using GraphPad Prism 5.0 software.

Results

In Vitro Studies

After exposure to low serum (1 % FBS) or TNF alpha for 48 h, MSCs displayed increased oxidative stress conversion of 2’7’-dichlorodihydrofluorescein diacetate (H₂DCFDA) to 2’7’-dichlorofluorescein compared with control conditions (10 % FBS) (Fig. 2a, left panel). Both stressors induced cellular cytotoxicity as assessed by measuring the lactose dehydrogenase (LDH) released into the media 72 h after cell treatment (Fig. 2a, right panel). At the protein level, the low serum stimulated the upregulation of proteins (p67^{phox} and XDH) involved in producing reactive oxygen species (ROS) (Fig. 2b). Importantly, the addition of 50 μM aliskiren in the medium was able to reduce the oxidative stress and prevent cellular cytotoxicity (Fig. 2a), normalizing the expression of p67^{phox} and XDH proteins (Fig. 2b).

In Vivo Studies

Importantly, the dose of aliskiren (15 mg/kg body weight/day) delivered to the mice (IA and IMA groups) did not significantly affect the SBP levels even 10 days after the subcutaneous implantation of the osmotic pump when compared to I and IM groups (baseline 115.5 ± 1.197 mmHg, I 101.9 ± 3.396 mmHg, IA 93.69 ± 4.277 mmHg, IM 101.1 ± 2.195 mmHg, IMA 96 ± 0.9774 mmHg, $p=0.1351$ comparing the groups of mice with myocardial infarction).

After the transepical infusion of mMSCs into the ischemic region, a strong Fluc activity was detected over the heart, but not in other organs, for the duration of the study. At 3 and 7 days, this signal was significantly higher in those mice that received aliskiren (IMA group), when compared to the animals that did not (IM group, Fig. 3a, b).

Furthermore, 10 days after the induction of myocardial infarction, only the animals in the IMA group showed echocardiographic evidence of significantly improved systolic function, as indicated by the decrease in end-systolic volume and the increase in ejection fraction (Fig. 4a, b) compared with the I group mice.

Ex Vivo Studies

The ischemic myocardium displayed histological changes of inflammatory cell infiltration and extracellular matrix deposition (Fig. 5a), which was significantly reduced in the IMA group mice compared with I and IM groups (Fig. 5b). Importantly, wall thickness measurements in hematoxylin and eosin (H&E)-stained sections revealed a significant increase in wall thickness for those animals that received both mMSCs and the drug aliskiren compared with untreated animals (Fig. 5b).

Furthermore, immunohistochemical staining of myocardial tissues showed a significant decrease in the upregulation of the inflammatory marker NFkB, in both the ischemic and the peri-ischemic area of IA and IMA mice compared to I mice (Fig. 6a, b). This result was associated with a significant increase in capillary density (vWf-positive staining) in the peri-infarct region of IMA mice compared to I mice (Fig. 6c, d). Finally, the expression of TGFb did not show any significant differences between all groups (data not shown).

Discussion

In this study, we showed that direct renin inhibition improves the survival of stem cells transplanted to the post-ischemic myocardium. Furthermore, the increased survival of MSCs translated to improved cardiac function.

Recently, cell-based intervention has appeared as a promising alternative for myocardial repair in CHD [6]. However, it has been argued that the survival of transplanted stem cells and therefore their reparative potential might be compromised by different noxious stimuli in the ischemic and peri-ischemic tissue [6, 8, 13, 20]. The modest although significant beneficial effects observed have inspired the development of preclinical studies aimed to improve the biology of the cells prior to their administration (by gene transfection [8, 21, 22], hypoxic preconditioning [19], pharmacological intervention [20]); however, modulation

of the microenvironment of the host tissue could represent an alternative approach. In the current study, we focused on the systemic regulation of the RAAS pathway via renin inhibition and, more importantly, on the effect that can have on the survival of transplanted stem cells in a mouse model of myocardial infarction.

To elucidate the mechanisms that play a role in stem cell survival, it is important to study cell survival in the in vivo setting. For that purpose, BLI has been shown to strongly correlate with the number of cells both in vitro [7] and in vivo [23]. Thus, optical bioluminescence imaging has been adopted in many preclinical studies to longitudinally monitor cell fate, especially in animal models of CHD [13, 24, 20, 23, 25]. Our study confirmed prior observations [5] with poor stem cell survival 24–48 h following delivery into the infarcted heart. Furthermore, we were able to observe a significant increase in MSC survival in those animals treated with the potent renin inhibitor, aliskiren. Not only was this improvement evident just 2 days after MSCs delivery in the ischemic myocardium, but also it was sustained throughout the entire duration of the study.

The role of MSCs in the long-term preservation of cardiac function after myocardial infarction remains to be elucidated [6, 26]. Several investigators reported modest improvements in systolic function after MSC transplantation, but more recent studies described these beneficial effects as short term [5, 7, 26, 27]. In our study, we did not observe any benefit in cardiac function after just 7 days following stem cells delivery. It should be taken into account that issues like cell type, together with the timing and conditions of delivery, could account for these differences. In addition, it has been suggested that the poor cell survival of MSCs might underlie this short-term effect, limiting a more sustained therapeutic action of these cells [8]. In fact, Mangi et al. reported that genetic modification of MSCs with Akt gene, a general mediator of survival signals, could preserve their viability in the early post-transplant period and, therefore, enhance their ability to restore left ventricular systolic performance [8]. In this study, we show that modulation of the post-ischemic myocardium improved MSC survival that was associated with partially preserved wall thickness and an improvement in cardiac function. The improvement in cardiac function was mainly due to a significant decrease in end-systolic volumes, probably suggesting better contractile conditions, while diastolic volumes remained unchanged. These results emphasize the importance of improving cell survival in the success of cell-based regenerative strategies.

The improvement in the survival of MSCs was independent of any effect on blood pressure. In this study, we used a dose of aliskiren that did not affect the systolic blood pressure of the animals 10 days after the induction of myocardial infarction. Furthermore, in our model and with the amount of aliskiren used, we could not observe any beneficial effect on cardiac function. Thus, we could postulate that the significant improvements observed were mainly due to the therapeutic effect of the survived cells.

The histological analysis revealed that, in our study, aliskiren could have played an important role in cardiac remodeling, decreasing the degree of inflammation and preventing the accumulation of collagen in the scar tissue post-ischemia. Previous studies have shown that MMP9, which is increased in the post-ischemic myocardium and is associated with

tissue inflammation, is normalized by treatment with aliskiren [16]. Furthermore, we believe that direct renin inhibition can act as a co-adjuvant to prepare a more “welcoming” post-ischemic myocardium, leading to better survival of transplanted stem cells. Although direct renin inhibition aided in preventing a pro-inflammatory response in the post-ischemic myocardium, it is important to note that only the group of mice that received both aliskiren and MSCs displayed a significant decrease in fibrosis as well as a significant increase in number of capillaries in the peri-infarct area. Therefore, we could speculate that the better survival of transplanted MSCs not only prevented the pro-fibrotic process, but also promoted myocardial perfusion, leading to a significant improvement in cardiac function. In this study, MSCs were delivered to the border (penumbra) zone but the beneficial effect was observed not only in the border zone but also in the infarcted area. Although it is possible that a few of the MSCs could have migrated to the infarcted area, a more plausible explanation is that MSCs, through their paracrine effect, modulated the post-ischemic myocardium limiting the infarct area.

Furthermore, the in vitro studies performed with mouse MSCs have shown that different stress conditions—either low serum or TNF alpha, one of the pro-inflammatory cytokines released in the ischemic heart—led to an increase in ROS (conversion of DCHFDA) that was associated with an increase in cellular toxicity (LDH release) and a significant upregulation of pro-oxidant proteins. The fact that aliskiren was able to preserve cell biology in vitro suggests a possible modulation of oxidative stress in the in vivo setting, which may have led to a better survival of transplanted cells. As a matter of fact, we have previously shown that oxidative stress plays an important role in the noxious environment after MI [13, 20].

In the current study, the benefit of aliskiren on MSC survival was limited to the initial 7–10 days post-MI. Several aspects should be considered as potential explanations to this limited effect. On one side, it is possible that the modulation strategy is not optimal, in agent or dose, for the noxious post-ischemic myocardial microenvironment. Furthermore, we cannot exclude that other factors can play a role in this setting. Although modulation of the RAAS has been shown in part to modulate oxidative stress [28, 29], it may not been the optimal strategy by itself. In the future, a combination of modulation strategies targeted to modulate other myocardial pathways (e.g., fibrosis) and directly improve stem cell biology may prove more efficacious. Furthermore, the amount of cells used in this study may not represent the optimal MSC-myocardium ratio for the best reparative effect post-ischemia. Future studies should focus on some of these variables to further improve the survival and efficacy of stem cells after transplantation.

In this study, we have shown that renin inhibition of the post-infarcted myocardial microenvironment, independent of changes in blood pressure, improves the survival and efficacy of transplanted MSCs. Strategies that focus on the myocardial microenvironment can enhance the engraftment of stem cells in the ischemic heart, which may eventually lead to greater improvements in cardiac function.

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References

1. Gersh BJ, Simari RD, Behfar A, Terzic CM, Terzic A. Cardiac cell repair therapy: a clinical perspective. *Mayo Clinic Proceedings*. 2011; 84(10):876–892. DOI: 10.4065/84.10.876 [PubMed: 19797777]
2. Assmus B, Honold J, Schächinger V, Britten MB, Fischer-Rasokat U, Lehmann R, et al. Transcatheter transplantation of progenitor cells after myocardial infarction. *The New England Journal of Medicine*. 2006; 355(12):1222–1232. DOI: 10.1056/NEJMoa051779 [PubMed: 16990385]
3. Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Hölschermann H, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *The New England Journal of Medicine*. 2006; 355(12):1210–1221. DOI: 10.1056/NEJMoa060186 [PubMed: 16990384]
4. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet*. 2006; 367(9505):113–121. DOI: 10.1016/S0140-6736(05)67861-0 [PubMed: 16413875]
5. van der Bogt KEA, Sheikh AY, Schrepfer S, Hoyt G, Cao F, Ransohoff KJ, et al. Comparison of different adult stem cell types for treatment of myocardial ischemia. *Circulation*. 2008; 118(14_suppl_1):S121–S129. DOI: 10.1161/CIRCULATIONAHA.107.759480 [PubMed: 18824743]
6. Mazo M, Araña M, Pelacho B, Prósper F. Mesenchymal stem cells and cardiovascular disease: a bench to bedside roadmap. *Stem Cells International*. 2012; 2012:1–11. DOI: 10.1155/2012/175979
7. van der Bogt KEA, Schrepfer S, Yu J, Sheikh AY, Hoyt G, Govaert JA, et al. Comparison of transplantation of adipose tissue- and bone marrow-derived mesenchymal stem cells in the infarcted heart. *Transplantation*. 2009; 87(5):642–652. DOI: 10.1097/TP.0b013e31819609d9 [PubMed: 19295307]
8. Mangi AA, Noisieux N, Kong D, He H, Rezvani M, Ingwall JS, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nature Medicine*. 2003; 9(9):1195–1201. DOI: 10.1038/nm912
9. Mishra PK. Bone marrow-derived mesenchymal stem cells for treatment of heart failure: is it all paracrine actions and immunomodulation? *Journal of Cardiovascular Medicine (Hagerstown, Md.)*. 2008; 9(2):122–128. DOI: 10.2459/JCM.0b013e32820588f0
10. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *Journal of the American College of Cardiology*. 2009; 54(24):2277–2286. DOI: 10.1016/j.jacc.2009.06.055 [PubMed: 19958962]
11. Chen, S-l; Fang, W-w; Ye, F.; Liu, YH.; Qian, J.; Shan, S-j, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *The American Journal of Cardiology*. 2004; 94(1):92–95. DOI: 10.1016/j.amjcard.2004.03.034 [PubMed: 15219514]
12. Forrester JS, Makkar RR, Marban E. Long-term outcome of stem cell therapy for acute myocardial infarction: right results, wrong reasons. *Journal of the American College of Cardiology*. 2009; 53(24):2270–2272. DOI: 10.1016/j.jacc.2009.03.023 [PubMed: 19520250]
13. Psaltis PJMP, Peterson KMB, Xu RM, Franchi FP, Witt TC, Chen IYMP, et al. Noninvasive monitoring of oxidative stress in transplanted mesenchymal stromal cells. *Journal of the American College of Cardiology: Cardiovascular Imaging*. 2013; 6(7):795–802. DOI: 10.1016/j.jcmg.2012.11.018 [PubMed: 23643284]
14. Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. *Circulation Research*. 2012; 110(1):159–173. DOI: 10.1161/CIRCRESAHA.111.243162 [PubMed: 22223212]
15. Perlini S, Salinaro F, Fonte ML. Direct renin inhibition: another weapon to modulate the renin-angiotensin system in postinfarction remodeling? *Hypertension*. 2008; 52(6):1019–1021. DOI: 10.1161/HYPERTENSIONAHA.108.121590 [PubMed: 18955659]

16. Westermann D, Riad A, Lettau O, Roks A, Savvatis K, Becher PM, et al. Renin inhibition improves cardiac function and remodeling after myocardial infarction independent of blood pressure. *Hypertension*. 2008; 52(6):1068–1075. DOI: 10.1161/HYPERTENSIONAHA.108.116350 [PubMed: 18955663]
17. Solomon SD, Shin SH, Shah A, Skali H, Desai A, Kober L, et al. Effect of the direct renin inhibitor aliskiren on left ventricular remodelling following myocardial infarction with systolic dysfunction. *European Heart Journal*. 2011; 32(10):1227–1234. DOI: 10.1093/eurheartj/ehq522 [PubMed: 21317148]
18. Soleimani M, Nadri S. A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. *Nature Protocols*. 2009; 4(1):102–106. DOI: 10.1038/nprot.2008.221 [PubMed: 19131962]
19. Peterson KM, Aly A, Lerman A, Lerman LO, Rodriguez-Porcel M. Improved survival of mesenchymal stromal cell after hypoxia preconditioning: role of oxidative stress. *Life Sciences*. 2011; 88(1–2):65–73. DOI: 10.1016/j.lfs.2010.10.023 [PubMed: 21062632]
20. Rodriguez-Porcel M, Gheysens O, Paulmurugan R, Chen IY, Peterson KM, Willmann JK, et al. Antioxidants improve early survival of cardiomyoblasts after transplantation to the myocardium. *Molecular Imaging and Biology: The Official Publication of the Academy of Molecular Imaging*. 2010; 12(3):325–334. DOI: 10.1007/s11307-009-0274-4
21. Wang X, Zhao T, Huang W, Wang T, Qian J, Xu M, et al. Hsp20-engineered mesenchymal stem cells are resistant to oxidative stress via enhanced activation of Akt and increased secretion of growth factors. *Stem Cells*. 2009; 27(12):3021–3031. DOI: 10.1002/stem.230 [PubMed: 19816949]
22. Tsubokawa T, Yagi K, Nakanishi C, Zuka M, Nohara A, Ino H, et al. Impact of anti-apoptotic and anti-oxidative effects of bone marrow mesenchymal stem cells with transient overexpression of heme oxygenase-1 on myocardial ischemia. *American Journal of Physiology. Heart and Circulatory Physiology*. 2010; 298(5):H1320–H1329. DOI: 10.1152/ajpheart.01330.2008 [PubMed: 20154257]
23. Rodriguez-Porcel M, Gheysens O, Chen IY, Wu JC, Gambhir SS. Image-guided cardiac cell delivery using high-resolution small-animal ultrasound. *Molecular Therapy: The Journal of the American Society of Gene Therapy*. 2005; 12(6):1142–1147. DOI: 10.1016/j.ymthe.2005.07.532 [PubMed: 16111921]
24. Wu JC. Molecular imaging of cardiac cell transplantation in living animals using optical bioluminescence and positron emission tomography. *Circulation*. 2003; 108(11):1302–1305. DOI: 10.1161/01.CIR.0000091252.20010.6E [PubMed: 12963637]
25. Psaltis PJ, Simari RD, Rodriguez-Porcel M. Emerging roles for integrated imaging modalities in cardiovascular cell-based therapeutics: a clinical perspective. *European Journal of Nuclear Medicine and Molecular Imaging*. 2011; 39(1):165–181. DOI: 10.1007/s00259-011-1925-7 [PubMed: 21901381]
26. Dai W, Hale SL, Martin BJ, Kuang JQ, Dow JS, Wold LE, et al. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. *Circulation*. 2005; 112(2):214–223. DOI: 10.1161/CIRCULATIONAHA.104.527937 [PubMed: 15998673]
27. Amsalem Y, Mardor Y, Feinberg MS, Landa N, Miller L, Daniels D, et al. Iron-oxide labeling and outcome of transplanted mesenchymal stem cells in the infarcted myocardium. *Circulation*. 2007; 116(11 Suppl):I38–I45. DOI: 10.1161/CIRCULATIONAHA.106.680231 [PubMed: 17846324]
28. Poss J, Werner C, Lorenz D, Gensch C, Bohm M, Laufs U. The renin inhibitor aliskiren upregulates pro-angiogenic cells and reduces atherogenesis in mice. *Basic Research in Cardiology*. 2010; 105(6):725–735. DOI: 10.1007/s00395-010-0120-5 [PubMed: 20857126]
29. Singh VP, Le B, Khode R, Baker KM, Kumar R. Intracellular angiotensin II production in diabetic rats is correlated with cardiomyocyte apoptosis, oxidative stress, and cardiac fibrosis. *Diabetes*. 2008; 57(12):3297–3306. DOI: 10.2337/db08-0805 [PubMed: 18829990]

Fig. 1.

Characterization of mouse mesenchymal stromal cells (mMSCs). MSCs at passage 4 were analyzed by fluorescent-activated cell sorting (FACS). Cells were CD45⁻ (hematopoietic marker), CD90⁺, CD29⁺, CD73⁺, and CD44⁺, markers characteristic of MSCs

Fig. 2.

In vitro studies. **a** Assessment of the oxidant status and cellular toxicity of MSCs after exposure to low serum or TNF alpha with or without aliskiren. Data represent mean $\pm$ SEM. * p <0.05; *** p <0.001 vs. each challenge without aliskiren. **b** Western blot analysis of

NAD(P)H oxidase p67^{phox} and Xanthine dehydrogenase (*XDH*), showing that aliskiren is able to normalize the expression of proteins involved in the production of reactive oxygen species. Data represent mean $\pm$ SEM. * p <0.05; ** p <0.01 vs. 10 % FBS, § p <0.05 vs. 1 % FBS

Fig. 3.

In vivo bioluminescent imaging (BLI). **a** Representative BLI images (7 days after surgery) of a mouse+MSCs (*IM*, *left panel*) and a mouse+MSCs and aliskiren (*IMA*, *right panel*). The signal due to MSCs engraftment localized over the heart region (*red arrows*), while the signal due to the osmotic pump material localized on the flank where the pump was installed (*blue arrows* and *magnification*). **b** As the BLI signal strongly correlates with the number of cells, the percentage of living cells has been plotted for both groups, considering the signal obtained at day 1 as a reference (100 % viability). Data represent mean±SEM. * $p<0.05$ vs. mice+MSCs

Fig. 4.

Left ventricular (LV) systolic function assessment. **a** Representative M-mode echocardiographic acquisitions 10 days after induction of myocardial infarction by LAD ligation. *AW* anterior wall, *LV* left ventricle, *PW* posterior wall. **b** Analysis of systolic function parameters, including LV end-systolic volume, LV end-diastolic volume, and LV ejection fraction. Data represent mean $\pm$ SEM. * $p<0.05$ vs. I group. *BL* baseline, *I* myocardial infarction, *IA* myocardial infarction+aliskiren, *IM* myocardial infarction+MSCs, *IMA* myocardial infarction+MSCs and aliskiren

Fig. 5. Histological analysis. **a** Representative images of histological staining of myocardium: hematoxylin and eosin (*H&E, top panel*) and picrosirius red (*bottom panel*). Gray arrows are pointing to the infarcted areas. **b** Analysis of the myocardial wall thickness in the infarcted region and the percentage of fibrotic tissue. Data represent mean±SEM. * $p<0.05$ vs. I group. $^{\S}p<0.05$ vs. IM group

Fig. 6.

a Representative images ($\times 20$) of NFκB staining of the border (*top panel*) and infarcted (*bottom panel*) region of the myocardium. **b** Analysis of the expression of NFκB in the border (*left*) and infarcted (*right*) region of the myocardium, expressed as percentage of positive stained area. **c** Representative images ($\times 20$) of vWf staining of the peri-infarct region of the myocardium. **d** Analysis of the expression of vWf, expressed as total number

of capillaries per field of view. Data represent mean $\pm$ SEM. * $p<0.05$; ** $p<0.01$ vs. I group. §§ $p<0.01$ vs. IM group

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