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Treatment with granulocyte-colony stimulating factor in patients with acute myocardial infarction. Evidence for a stimulation of neovascularization and improvement of myocardial perfusion

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Received December 6, 2005, accepted January 26, 2006

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Pharmazie 61: 957–961 (2006)

Background: Stem cell therapy has been suggested to be beneficial in patients after acute myocardial infarction (AMI). Strategies of treatment are either a local application of mononuclear bone marrow cells (BMCs) into the infarct-related artery or a systemic therapy with the granulocyte-stimulating factor (G-CSF) to mobilize BMCs. Nevertheless, the mechanisms responsible for improvement of cardiac function and perfusion are speculative at present. This study has been performed to investigate the effect of G-CSF on systemic levels of vascular growth factors and chemokines responsible for neovascularization, that might help to understand the positive effects of a G-CSF therapy after AMI.
Methods and Results: Five patients in the treatment group and 5 patients in the control group were enrolled in this study. The patients in the treatment group received 10 μg/kg bodyweight/day of G-CSF subcutaneously for a mean treatment duration of 6.6 ± 1.1 days. In both groups, levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and monocyte chemotactic protein-1 (MCP-1) were measured on day 2 to 3 and day 5 after AMI. The regional wall perfusion and the ejection fraction (EF) were evaluated before discharge and after 3 months with ECG-gated MIBI-SPECT and radionuclide ventriculography, respectively. Significant higher levels of VEGF ($p < 0.01$), bFGF ($p < 0.05$) and MCP-1 ($p < 0.05$) were found in the treatment group compared to the control group. Levels of VEGF and bFGF remained on a plateau during the G-CSF treatment and decreased significantly in the control group. The wall perfusion improved significantly within the treatment group and between the groups ($p < 0.05$), respectively. The EF improved significantly within the treatment group ($p < 0.05$), but the change of the EF between the groups was not significant.
Conclusion: In patients with AMI, the treatment with G-CSF modulates the formation of vascular growth factors that might improve neovascularization and result in an improved myocardial perfusion and function.

1. Introduction

Occlusion of coronary arteries leads to necrosis of myocytes and vascular structures within hours in the myocardium perfused by the artery. Despite percutaneous coronary intervention or thrombolysis or both to reopen the occluded vessel, loss of myocytes and vascular structures after myocardial infarction cannot be avoided. A regeneration of cardiomyocytes and stimulation of neovascularization by means of stem cells is a promising approach to prevent further loss of myocardium and cardiac function. Several clinical investigations have been done to demonstrate an effect of stem cell therapy on myocardial function by intracoronary application of mononuclear bone marrow cells (BMCs) with various results (Strauer et al. 2002; Assmus et al. 2002; Wollert et al. 2004; Kuethe et al. 2004b). A novel approach of stem cell therapy after acute myocardial infarction by mobilization of BMCs by treatment with the granulocyte-colony stimulating factor (G-CSF) showed a positive effect on global left ventricular function as well as on regional wall motion and perfusion (Kuethe et al. 2004a, 2005). Nevertheless, all of these clinical studies can only show a relationship between a so-called stem cell therapy and a positive effect on cardiac function, but the mechanisms are still under discussion. At present, it is not clear whether cells from the bone marrow are able to transdifferentiate into cells of the myocardium, or stem cells fuse with resident cells in the myocardium, or a stimulation of neovascularization and angiogenesis by means of angiogenic cytokines and chemokines is responsible for the positive effects of these therapies (Balsam et al. 2004; Murry et al. 2004; Norol et al. 2003). The focus of the present investigation was the potential effect of G-CSF treatment on stimulation of vascular growth fac-

tors and chemokines known to be involved in neovascularization or angiogenesis. This may help to understand the positive effects of a treatment with G-CSF after acute myocardial infarction in humans.

2. Investigations and results

The characteristics of the verum and control patients including demographic, clinical, angiographic and laboratory data are shown in the Table. There were no significant differences between the groups. In all patients, a complete reperfusion was achieved and the residual stenosis in the target vessel after stent implantation was below 10%. The patients received either tirofiban or eptifibatide for 24 h. The mean time of treatment with G-CSF was 6.6 ± 1.1 days. The treatment with G-CSF resulted in a 4-fold increase of whole blood leucocytes (14.9 ± 8.1 vs. 61.7 ± 8.9 cells* 10^9/l; $p < 0.05$) and a 20-fold increase of $CD34^+$ cells (4.2 ± 1.6 vs. 84.7 ± 64.8 cells/µl; $p < 0.05$). The peak $CD34^+$ cell count was reached after 4.6 ± 0.5 days.

To find out whether there is a regulation of VEGF, bFGF and MCP-1 as a consequence of an acute myocardial infarction, the levels of VEGF, bFGF and MCP-1 were compared between the control group 2 to 3 days after AMI and a healthy control group. Levels of VEGF and bFGF were significantly increased after AMI compared to healthy controls, but this was not true for MCP-1 (Fig. 1).

In Fig. 2 we demonstrate the time course of the concentrations of VEGF, bFGF and MCP-1 in the control and the verum group. At least one injection of G-CSF leads to an increase of VEGF and MCP-1 concentrations, but does not modulate bFGF levels. Levels of VEGF, bFGF and MCP-1 fell significantly from day 2 to 3 after AMI to day 5 after AMI in the control group, whereas in the verum group, VEGF levels remained constant from day 2 to 3 to day 5 after AMI, bFGF levels increased and MCP-1 levels decreased slightly, but significantly (Fig. 2). A comparison between the verum and the control group at day 5 after AMI showed a highly significant increase regarding VEGF levels, and a significant increase of bFGF and MCP-1 levels (Fig. 2).

Baseline examinations started in all patients with ^{99m}Tc-MIBI SPECT imaging after 8.6 ± 1.7 days in the verum group and $7.8 \pm$ in the control group. The ejection fraction in the verum group increased significantly from baseline to 3-month follow-up by 4.4% (from $44.8 \pm 10.7\%$ to $49.2 \pm 12.9\%$; $p < 0.05$). In the control group the ejection fraction increased not significantly by 3.8% (from $38.8 \pm 15.1\%$ to $42.6 \pm 13.1\%$; $p = 0.20$). The changes between the groups were not significantly different (Fig. 3). In the verum group the wall perfusion score showed a significant improvement from 15.8 ± 2.3 to 11.8 ± 1.3 ($p < 0.05$). There was no significant difference of the wall perfusion score in the control group ($p = 0.79$). Between the groups the change of the wall perfusion score was significant ($p < 0.05$).

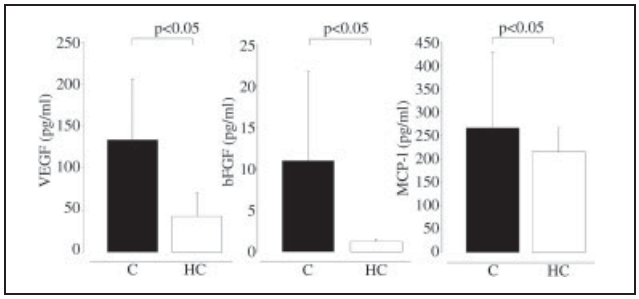

Fig. 1: Comparison of serum levels of VEGF, bFGF and MCP-1 between the control group (C) 2 to 3 days after acute myocardial infarction and healthy controls (HC) with no known history of coronary heart disease. The factors were measured by ELISA. P values reflect comparison between the groups (see Experimental)

3. Discussion

The present study demonstrates that the treatment of patients with G-CSF suffering from an AMI leads to a modulation of VEGF-, bFGF- and MCP-1-levels compared to a standard treatment. This modulation is associated with an improvement of the myocardial perfusion and the left ventricular function. As we have shown previously, the treatment with G-CSF in patients with AMI is feasible and safe and does not lead to severe side effects (Kuethe et al. 2005).

The effect of a local treatment with mononuclear cells from the unstimulated bone marrow to improve cardiac function after AMI has been doubted, since experimental

Table: Characteristics of the patients in the verum and control group

	Verum (n = 5)	Control (n = 5)	P
Age (y)	56.6 ± 16.2	55.8 ± 16.2	0.94
Sex (male/female)	5/0	4/1	0.29
Body mass index (kg/m^2)	29.9 ± 4.7	28.3 ± 5.2	0.62
Diabetes	0	1	0.34
Hyperlipidaemia	2	1	0.43
Smoking	2	3	0.76
Symptom onset to angioplasty time (hrs)	4.4 ± 1.3	6.4 ± 3.9	0.32
Infarct-related vessels (LAD/RCA/LCX)	3/2/0	4/1/0	0.64
CK max (µmol/l, normal value: 0–1.33)	32.5 ± 19.8	31.8 ± 17.4	0.21
Leucocytes (cells* 10^9/l) prior to therapy	14.9 ± 8.1	12.3 ± 2.8	0.52
C-reactive protein (mg/l) prior to therapy	6.4 ± 2.9	5.8 ± 3.0	0.78
Duration of G-CSF treatment (days)	6.6 ± 1.1	–	–
Medication on discharge:			
Aspirin	5/5	5/5	1.0
Clopidogrel	5/5	5/5	1.0
ACE inhibitor	5/5	5/5	1.0
β-Blocker	5/5	5/5	1.0
Statin	5/5	5/5	1.0

LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; CK, creatine kinase; G-CSF, granulocyte-colony stimulating factor

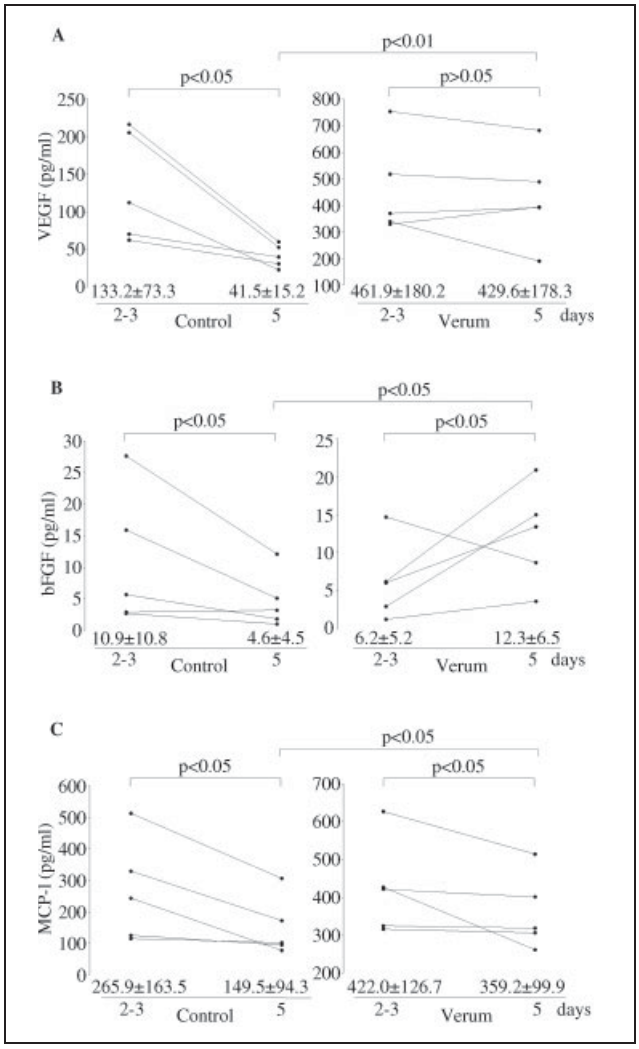

Fig. 2: Serum levels of VEGF (A), bFGF (B) and MCP-1 (C) measured by ELISA 2 to 3 days after acute myocardial infarction and after 5 days. At day 5, patients in the treatment group were given G-CSF for 3 days. P values reflect comparison within the groups and between the groups at day 5 (see Experimental)

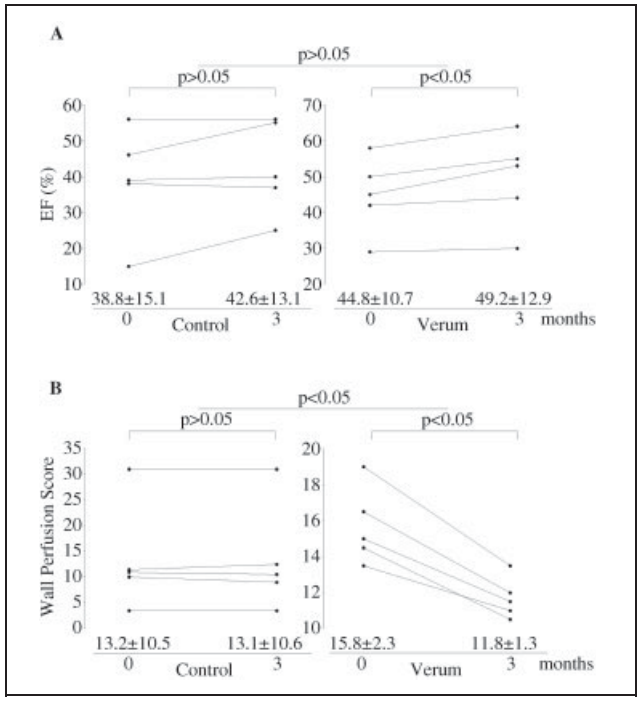

Fig. 3: Ejection fraction (A) and wall perfusion score (B) measured by radionuclidventriculography and by ECG gated Sestamibi SPECT before discharge and at 3-months follow-up. All measurements were performed at rest. P values reflect comparison within the groups and between the groups over time (see Experimental). The decrease of the score reflects an improvement of the wall perfusion

studies have shown that BMCs did not transdifferentiate into cells of the myocardium (Balsam et al. 2004; Murry et al. 2004), and a clinical study in patients with a large anterior myocardial infarction showed no benefit (Kuethe et al. 2004b). On the other hand, the treatment with G-CSF does not only stimulate and mobilize stem cells from the bone marrow, but has pleiotropic effects on remodelling processes and neovascularization, that leads to an improvement of myocardial perfusion and function.

Vascular endothelial growth factor (VEGF) and its receptor have been shown to be critical in the development of cardiomyopathy. In human idiopathic dilated cardiomyopathy, VEGF mRNA levels and mRNA of the VEGF receptor have been shown to be downregulated (Abraham et al. 2000). Yoon et al. could demonstrate in a diabetic rat model, that a downregulation of myocardial VEGF expression precedes all other features of dilated cardiomyopathy. It was possible to influence the native course of the cardiomyopathy in this model by restoration of the myocardial VEGF expression via local gene therapy. This restores the myocardial microcirculation and cardiac function by increasing the count of circulating endothelial progenitor cells and its homing into the myocardium, and by decreasing the apoptosis of endothelial cells and cardiomyocytes (Yoon et al. 2005). A recently published work by Giordano et al., who showed a paracrine secretion of VEGF by cardiomyocytes, underscores the above mentioned importance of VEGF and its receptor in the maintenance of cardiac function. The group demonstrated that a cardiomyocyte-specific knockout of the VEGF gene leads to fewer coronary microvessels, thinned ventricular walls and a depressed basal and stimulated contractile function (Giordano et al. 2001). By means of a VEGF gene transfer alone the cardiac function in chronic myocardial ischemia can be improved, but the effect of gene therapy can be increased by a cytokine therapy with G-CSF and a consecutive mobilization of endothelial progenitor cells incorporated into the ischemic myocardium (Kawamoto et al. 2004). For the homing of stem cells into the myocardium, stromal cell derived factor-1 (SDF-1) seems to be essential. In a chronic ischemic model, homing of stem cells can be restored by SDF-1 gene therapy. Immediately after AMI, SDF-1 is upregulated and will be downregulated within the following 7 days (Askari et al. 2003). Therefore, a mobilization of stem cells via treatment with G-CSF in humans can be sufficient and should be performed as soon as possible after AMI, possibly earlier than 48 h after AMI.

Not only endothelial progenitor cells, but also mesenchymal stem cells can integrate into the border zone of the infarcted myocardium and improve cardiac function by enhancing angiogenesis and myogenesis after AMI in rats (Nagaya et al. 2004). G-CSF is known to mobilize mesenchymal stem cells after AMI in mice, and the quantity of mobilized mesenchymal stem cells can be calculated by measurement of $CD34^+$ cells (Kawada et al. 2004). We speculate that mesenchymal stem cells are also mobilized in a considerable amount in our study population. Mesenchymal stem cells enhance angiogenesis partly by increasing endogenous levels of VEGF and VEGF receptor. Therefore, mesenchymal stem cells contribute to neovas-

cularization through a growth factor-mediated paracrine regulation (Chen et al. 2003). Finally, a phase I study of intracoronary application of recombinant human VEGF in patients with chronic ischemia showed an improvement of myocardial perfusion at rest 60 days after treatment, but only in the high dose group (Hendel et al. 2000).

FGF and MCP-1 have also been stimulated under treatment with G-CSF in our study. In a randomised study, FGF was given in 40 patients undergoing coronary artery bypass grafting as intramyocardial injections with active or heat-denaturated protein around the distal left anterior descending artery. Twelve weeks later the authors found a pronounced contrast accumulation angiographically only in the group of patients treated with the active growth factor (Schumacher et al. 1998). In an open label phase I study of FGF-2 administered as a single 20-min infusion in patients with ischemic heart disease, the authors demonstrated an improvement in quality of life and in exercise tolerance. Furthermore, the magnetic resonance imaging showed increased regional wall thickening and a reduction in the extent of the ischemic area (Laham et al. 2000).

MCP-1 is a critical chemokine in neovascularization exerting its effects by mediating the invasion of monocytes supporting the inflammatory reaction after acute ischemia. The monocytes, in turn, are able to produce growth factors that lead to the proliferation of endothelial and smooth muscle cells. Ito et al. could demonstrate in a hindlimb ischemia model in rabbits, that a local infusion of MCP-1 increases both collateral and peripheral conductance after femoral artery occlusion due to enhanced vessel growth (Ito et al. 1997). This underscores the important role of monocytes in collateral growth and capillary sprouting that may prevent further ischemia by improvement of regional perfusion, possibly also in the myocardium.

At last, G-CSF may exert its beneficial effects by the inhibition of apoptotic pathways in endothelial cells and cardiomyocytes via activating the Jak/Stat pathway. This results in an improvement of cardiac function and increase of vascularization in infarcted hearts (Harada et al. 2005).

The benefit of G-CSF on top of a standard medical therapy in patients with acute myocardial infarction might be a consequence of 1. a stimulation of growth factors and chemokines 2. the mobilization of stem cells, especially of mesenchymal stem cells and endothelial progenitor cells and 3. the inhibition of apoptotic pathways. This results in an improvement of ventricular function and myocardial perfusion, as shown in our treatment group. The multiple sites of action of G-CSF possibly make this approach of therapy superior to the local application of mononuclear bone marrow cells. However, this idea seems very attractive, but it is speculative at present. Our preliminary data should be verified in a randomised trial.

4. Experimental

4.1. Patients

In a prospective, nonrandomized and open-label study, we included 23 patients with a first transmural myocardial infarction according to World Health Organization criteria from June 2002 to June 2004. All patients were acutely treated by recanalization of the infarct-related coronary artery by balloon angioplasty and subsequent stent implantation under GIIb/IIIa blockade. Patients between 18 and 80 years of age were eligible for inclusion. Patients who refused treatment with G-CSF, but fulfilled the criteria for inclusion into the study, served as controls. Patients were excluded if one of the following criteria were met: multivessel coronary artery disease with the need of interventions of non infarct-related coronary arteries, cardiogenic shock, major bleeding requiring blood transfusion after percutaneous coronary intervention, alcohol or drug dependency, history of leucopenia, anaemia or thrombocytopenia, evidence for malignant diseases or hepatic and renal dysfunction. All patients were informed in detail about the treatment and diagnostic procedures and written informed consent was obtained from each patient. The local ethics committee of the Friedrich-Schiller-University, Jena, approved the study protocol. All patients left the hospital with standard medication consisting of aspirin, clopidogrel, ACE inhibitor, β-blocker and statin. From May 2003 we collected serum for the measurement of cytokines and growth factors in 10 of these patients, from 5 patients in the treatment group and 5 in the control group. Eight members of the medical staff of the Friedrich-Schiller-University Jena between 18 and 54 years of age (1 female, 7 male) served as healthy controls with no known history of a coronary artery disease.

4.2. G-CSF – Treatment protocol

Treatment with G-CSF (Filgastrim, Neupogen, Amgen) started 48 h after recanalization and stent implantation and was initiated with a dose of 5 µg/kg body weight subcutaneously two times a day. For the determination of the treatment duration, measurement of $CD34^+$ cells was performed every day by flow cytometry (FACS Calibur, Becton Dickinson) with directly conjugated antibodies (anti-human $CD34^+$-FITC, Becton Dickinson). The treatment was stopped when the peripheral leukocyte count was above 50×10^9/l or the $CD34^+$ cell count decreased under current stimulation with G-CSF. To reduce the risk of thromboembolic events, therapeutic anticoagulation with enoxaparin was performed during the time of treatment. Common side effects like increase of body temperature, headache or bone pain were treated with 0.5 g paracetamol orally.

The first time point for collection of serum samples was 2 to 3 days after successful recanalization. The second time point was 5 days after successful recanalization that was after a period of 2.5 to 3 days of G-CSF treatment.

4.3. ECG gated sestamibi SPECT and radionuclide ventriculography

For evaluation of myocardial perfusion and global ejection fraction, all patients underwent ^{99m}Technetium-sestamibi (^{99m}Tc-MIBI) SPECT imaging and radionuclide ventriculography before discharge as baseline measurements.

^{99m}Tc-MIBI (350MBq) was administered intravenously. One hour later, each patient was positioned under a 90° dual-head Gamma camera (E.cam, Siemens Medical Systems). Images were acquired using a step-and-shoot body contour mode over a 90° arc, starting at the 45° right anterior oblique projection and ending at the 45° left posterior oblique projection, for a total of 32 projections at 30s per projection. Images were gated at 8 frames per cardiac cycle. All projection images were acquired into 64×64 image matrices with a 1.0 acquisition zoom and a simultaneous transmission scan (profile scan). The SPECT projection data were corrected for attenuation using the transmission scan and were reconstructed using the ITW iterative reconstruction method (10 iterations) by applying a butterworth filter with a cut-off frequency of 0.32 and a filter order of 5.0. The gated SPECT images were analysed with the Cedar's Quantitative gated SPECT program (QGS).

After *in vivo* labelling of red blood cells with 10 MBq/kg body weight of technetium-99m, a planar radionuclide ventriculography in the left anterior oblique view was performed (E.cam, Siemens Medical Systems). Following equilibration of the radioactivity data were obtained in an ECG-gated mode with 32 frames per cardiac cycle, a 64×64 matrix, a low-energy high-resolution parallel-hole collimator and 6.5 million counts. The data were analysed with a semiautomatic ICON-program (Siemens).

4.4. SPECT Imaging analysis

For the analysis of myocardial wall perfusion, the left ventricle was divided according to the 17-segment model proposed by the American Heart Association (Cerqueira et al. 2002). The myocardial perfusion was assessed visually by two blinded and experienced observers independently using a four point scale for assessment of every segment as follows: 0, normal; 1, mildly reduced; 2, severely reduced; 3, absent uptake. Differences were resolved by consensus of the two observers. The perfusion score was calculated for each patient as the sum of all segments.

4.5. Follow-up examination

After 3 months ECG-gated sestamibi SPECT and radionuclide ventriculography were repeated to assess the ejection fraction and regional perfusion.

4.6. Immunoassay of chemokines and growth factors

Measurement of serum levels of the vascular endothelial growth factor (VEGF), the basic fibroblast growth factor (bFGF) and the monocyte chemotactic protein-1 (MCP-1) using an assay, that employs the quantitative sandwich enzyme immunoassay technique. The assays are commercially available from R&D Systems (Wiesbaden, Germany). The assays were carried out according to the manufactures manual.

4.7. Statistical analysis

Differences in demographic characteristics between the control and the treatment groups were assessed with the χ^2 tests and t tests. Each patient in the control and the verum group was used as his own control and changes between baseline and follow-up in the groups were assessed with the Wilcoxon-Signed-Rank test. Differences between the control group at baseline and healthy controls, and between the control and verum group at day 5 were assessed with the Mann-Whitney-U test. The changes over time between the groups regarding the ejection fraction and the wall motion score were assessed with the Mann-Whitney-U test. We considered a p value of less than 0.05 statistically significant.

References

Abraham D, Hofbauer R, Schafer R et al. (2000) Selective downregulation of VEGF-A(165), VEGF-R(1), and decreased capillary density in patients with dilative but not ischemic cardiomyopathy. Circ Res 87: 644–647.

Askari AT, Unzek S, Popovic ZB et al. (2003) Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 362: 697–703.

Assmus B, Schachinger V, Teupe C et al. (2002) Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 106: 3009–3017.

Balsam LB, Wagers AJ, Christensen JL et al. (2004) Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 428: 668–673. Epub 2004 Mar 2021.

Cerqueira MD, Weissman NJ, Dilsizian V et al. (2002) Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart: a statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation 105: 539–542.

Chen J, Zhang ZG, Li Y et al. (2003) Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ Res 92: 692–699. Epub 2003 Feb 2027.

Giordano FJ, Gerber HP, Williams SP et al. (2001) A cardiac myocyte vascular endothelial growth factor paracrine pathway is required to maintain cardiac function. Proc Natl Acad Sci USA 98: 5780–5785. Epub 2001 May 5781.

Harada M, Qin Y, Takano H et al. (2005) G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. Nat Med 11: 305–311. Epub 2005 Feb 2020.

Hendel RC, Henry TD, Rocha-Singh K et al. (2000) Effect of intracoronary recombinant human vascular endothelial growth factor on myocardial perfusion: evidence for a dose-dependent effect. Circulation 101: 118–121.

Ito WD, Arras M, Winkler B et al. (1997) Monocyte chemotactic protein-1 increases collateral and peripheral conductance after femoral artery occlusion. Circ Res 80: 829–837.

Kawada H, Fujita J, Kinjo K et al. (2004) Non-hematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood 104: 3581–3587. Epub 2004 Aug 5.

Kawamoto A, Murayama T, Kusano K et al. (2004) Synergistic effect of bone marrow mobilization and vascular endothelial growth factor-2 gene therapy in myocardial ischemia. Circulation 110: 1398–1405. Epub 2004 Aug 1330.

Küthe F, Figulla HR, Herzau M et al. (2005) Treatment with granulocyte colony-stimulating factor for mobilization of bone marrow cells in patients with acute myocardial infarction. Am Heart J 150: 115.

Küthe F, Figulla HR, Voth M et al. (2004a) [Mobilization of stem cells by granulocyte colony-stimulating factor for the regeneration of myocardial tissue after myocardial infarction]. Dtsch Med Wochenschr 129: 424–428.

Küthe F, Richartz BM, Sayer HG et al. (2004b) Lack of regeneration of myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans with large anterior myocardial infarctions. Int J Cardiol 97: 123–127.

Laham RJ, Chronos NA, Pike M et al. (2000) Intracoronary basic fibroblast growth factor (FGF-2) in patients with severe ischemic heart disease: results of a phase I open-label dose escalation study. J Am Coll Cardiol 36: 2132–2139.

Murry CE, Soonpaa MH, Reinecke H et al. (2004) Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 428: 664–668. Epub 2004 Mar 2021.

Nagaya N, Fujii T, Iwase T et al. (2004) Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. Am J Physiol Heart Circ Physiol 287: H2670–2676. Epub 2004 Jul 2629.

Norol F, Merlet P, Isnard R et al. (2003) Influence of mobilized stem cells on myocardial infarct repair in a nonhuman primate model. Blood 102: 4361–4368. Epub 2003 Aug 4328.

Schumacher B, Pecher P, von Specht BU et al. (1998) Induction of neoangiogenesis in ischemic myocardium by human growth factors: first clinical results of a new treatment of coronary heart disease. Circulation 97: 645–650.

Strauer BE, Brehm M, Zeus T et al. (2002) Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 106: 1913–1918.

Wollert KC, Meyer GP, Lotz J et al. (2004) Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 364: 141–148.

Yoon YS, Uchida S, Masuo O et al. (2005) Progressive attenuation of myocardial vascular endothelial growth factor expression is a seminal event in diabetic cardiomyopathy: restoration of microvascular homeostasis and recovery of cardiac function in diabetic cardiomyopathy after replenishment of local vascular endothelial growth factor. Circulation 111: 2073–2085.