Original Article

The Improvement of Myocardial Function by Granulocyte Colony Stimulating Factor Following Acute Anterior Myocardial Infarction: A Double Blind Placebo Controlled Study

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Background: In patients with acute myocardial infarction (AMI), reperfusion of the occluded infarct-related artery significantly improves acute and late clinical outcome. There is increasing evidence that transplantation of autologous stem cells improves cardiac function after AMI. For propagation of peripheral blood stem cells, application of granulocyte–colony stimulating factor (G-CSF) has been shown to be feasible, effective, and safe.
Methods: Ten patients in the treatment group and 10 patients in the control group were enrolled in this prospective, randomized controlled and double blind study. Two weeks after myocardial infarction that was followed by successful recanalization and stent implantation, the patients of the treatment group received 10 μg/kg body weight per day (divided BID) G-CSF subcutaneously for a maximum duration of 5.0 days. In both groups, ejection fraction was evaluated with echocardiography and cardiac perfusion scans 10 days and 6 months after myocardial infarction. The Tei index was measured by echocardiography.
Results: No severe side effects of G-CSF treatment were observed. There was no significant improvement of left ventricular ejection fraction when the G-CSF treated group was compared to the control group (P=0.821 for cardiac scan and P=0.705 for echocardiography). Changes in Tei index was not significant in the treatment group (P=0.815); however, it was significantly deteriorated in the control group (P=0.005).
Conclusion: In patients with acute anterior myocardial infarction, treatment with G-CSF, is feasible and safe and seems to be effective in improving global cardiac function without affecting the ejection fraction under clinical conditions.

Keywords: Granulocyte–Colony Stimulating Factor, Myocardial Infarction

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Introduction

Despite the development of several new therapeutic modalities, ischemic heart diseases remain one of the major causes of morbidity and mortality worldwide. A considerable proportion of these patients will develop chronic congestive heart failure, which carries a mortality of approximately 20% per year in symptomatic cases.

The process of complex architectural myocardial alterations, referred to as ventricular remodeling, is initiated following a large myocardial infarction. Acute destruction of myocardial cells and extracellular matrix induce early ventricular dilatation, which is associated with deleterious cardiac function in the late phase.[1] Replacement by collagen fibers plays an essential role in preserving the integrity of the infarcted tissue, protecting against an even more pronounced dilatation of the left ventricle (LV). Cytokines such as transforming growth factor (TGF)-β1 play major roles in this healing process and reparative fibrosis.[2] The infarct repair process, involving inflammation and collagen synthesis, is another important factor that affects ventricular remodeling. While an excessive inflammatory reaction in the infarcted myocardium leads to poor

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clinical outcomes, such as ventricular rupture or congestive heart failure,[3] anti-inflammatory therapy after MI, such as corticosteroid administration is associated with catastrophic results in both clinical and experimental settings due to delayed collagen accumulation and scar formation.

Necrotic tissue can be replaced by regeneration of cardiomyocytes and stimulation of neovascularization . Experimental studies have shown that bone marrow cells (BMCs) can differentiate into myocytes and endothelial cells.[4-6] Clinical investigations have demonstrated improvement in myocardial function after intracoronary application of mononuclear BMCs.[7-9] This approach, however, requires a second catheterization procedure. Furthermore, only a small number of cells with stem cell characteristics, capable of transdifferentiation, can be harvested from the unstimulated bone marrow.

Granulocyte colony stimulating factor (G-CSF), a 20-kDa glycoprotein, is known to induce granulopoiesis.[10] It was reported that G-CSF by way of transdifferentiation of bone marrow stem cells in combination with stem cell factor improved ventricular remodeling after MI through myocyte regeneration [5,6]. In addition, G-CSF has been described to affect the healing process by modulating the inflammatory reaction and collagen synthesis due to its immunoregulatory properties.[11-13] Therefore, especially in the early phase of MI, where intense inflammation occurs, it is possible that G-CSF imparts its beneficial effect on ventricular remodeling by modulating the inflammatory process other than myocyte regenration.

On the basis of experimental animal data it was shown that BMCs mobilized by G-CSF have a positive effect on myocardial regeneration.[8,9,14]

Previous studies have shown that due to acute presence of inflammation, G-CSF is not effective in the acute phase of myocardial infarction, so the latest suggestion is administration of G-CSF at least 7-10 days post MI.[3,15,16]

We hypothesized that administration of G-CSF after MI can prevent ventricular remodeling. We therefore investigated the feasibility and safety of G-CSF and its effects on left ventricular function and remodeling in patients with acute myocardial infarction.

Patients and Method

This is a prospective, randomized double-blind placebo controlled study of 20 patients with a first acute anterior wall ST elevation myocardial infarction (STEMI). STEMI was diagnosed from typical chest pain at rest lasting ≥30 minutes, the presence of cumulative ST-elevations of ≥0.4 mV in ≥2 contiguous leads from V1-V6 on a standard 12-lead ECG, and a significant rise in serum markers of myocardial infarction. Patients were aged from 30 to 80 years and enrolled into the study from June 2008 to February 2009. Patients were excluded from the study if one of the following criteria was met: multivessel coronary artery disease; cardiogenic shock; major bleeding requiring blood transfusion after percutaneous coronary intervention; evidence for malignant diseases; or hepatic and renal dysfunction and previous myocardial infarction. All patients were briefed in detail about the diagnostic and treatment procedures and all of them received oral and written information about the study before signing an informed consent for participating in the study which had already been approved by the local ethics committee of Shiraz University of Medical Sciences.

Eighteen patients (90%) were acutely treated by infusion of streptokinase, 1 patient (5%) underwent successful balloon angioplasty and subsequent stent implantation under glycoprotein IIb/IIIa blockade. However, one patient (5%) did not receive intensive treatment because of elapsing ≥ 12 hours from the onset of symptoms and having a transient ischemic attack in ≤ 3 months. Subsequently, the patients without acute treatment or treated with streptokinase underwent successful balloon angioplasty followed by stent implantation under glycoprotein IIb/IIIa blockade. However, one patient did not receive such a treatment because his angiography was in favor of coronary spasm which led to myocardial infarction.

All patients were discharged from the hospital with standard medication consisting of aspirin, clopidogrel (Plavix: Sanofi-Aventis, France), angiotensin converting enzyme (ACE) inhibitor, beta blocker, and statins.

The patients in the treatment group were rehospitalized after 2 weeks of acute myocardial infarction and treatment with G-CSF (Neupogen. Filgrastim Switzerland) was initiated with a dose of 5 µg/kg body weight subcutaneously two times a day for maximum 5 days. Daily WBC counts were determined and the treatment was stopped when the peripheral leukocyte count was higher than 50000/µL. To reduce the risk of thromboembolic events, prophylactic anticoagulation with enoxaparin was performed throughout treatment period.

Common side effects such as increase in body temperature, headache, or bone pain were treated

Table 1. Patients' characteristics in G-CSF effect on myocardial performance in 20 patients

Characteristics	Treatment	Control	P value
Age	50.6 ± 9.2	51.7 ± 15.5	0. 849
Male	7(70%)	9(90%)	0.291
Cases of hypertension	4(40%)	4(40%)	0.675
Cases of diabetes mellitus	1(10%)	2(20%)	0.500
Smoker	7(70%)	6(60%)	0.500
Cases of hyperlipidemia	3(30%)	2(20%)	0.500
History of previous IHD	2(20%)	4(40%)	0.314
Mean lag period post MI to PCI	3.9 days	4 days	0.842
Tei index at baseline	0.512	0.622	0.286
Ejection fraction based on scan post MI at baseline	42.8%	47%	0.842
Positive family history	6(60%)	3(30%)	0.185

with 0.5 g paracetamol administered orally. The patients were discharged after receiving the complete dose of G-CSF. Those in the control group were admitted to the hospital 2 weeks after MI and received normal saline infusion as placebo for 5 days.

In order to evaluate myocardial perfusion and global ejection fraction (EF), all patients underwent technetium 99m sestamibi (99mTc-MIBI) single-photon emission computed tomography (SPECT) imaging and echocardiography after successful percutaneous intervention as baseline measurements.

Technetium 99m sestamibi (350 MBq) was administered intravenously. After one hour, each patient was positioned under a 908 dual-head gamma camera (E.cam, Siemens Medical Systems, Erlangen, Germany). Images were obtained using a step-and-shoot body contour mode over a 908 arc, starting at the 458 right anterior oblique projections .They ended at the 458 left posterior oblique projection, for a total of 32 projections at 30 seconds per projection. Images were gated at 8 frames per cardiac cycle. All projected 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. This was achieved by using the transmission scan and was reconstructed by the iterative Wallis reconstruction method (10 iterations) and applying a Butterworth filter with a cutoff frequency of 0.32 and a filter order of 5.0. The gated SPECT images were analyzed with the Cedar's Quantitative gated SPECT program. The data were analyzed with a semiautomatic ICON program (Siemens). Complete echocardiography study was performed by a blinded cardiologist and 2 dimension, M mode, Doppler, and tissue Doppler study was performed for each patient after successful percutaneous intervention myocardial performance in LV (Tei index) was calculated by using

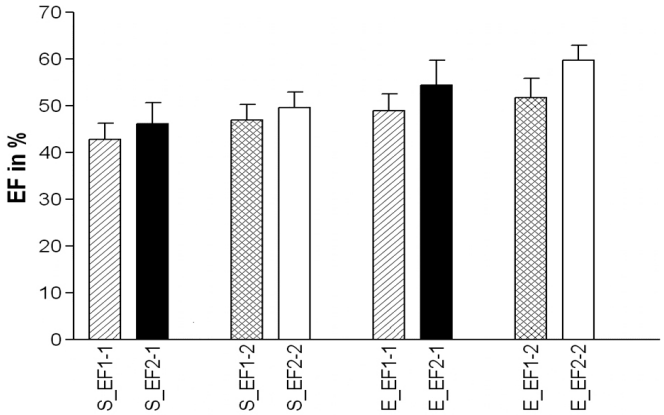

Figure 1. Ejection fraction results in control treatment groups. S: Scan; E: Echocardiography; EF1:1st ejection fraction; EF2:2nd ejection fraction; 1: Study group; 2: Control group

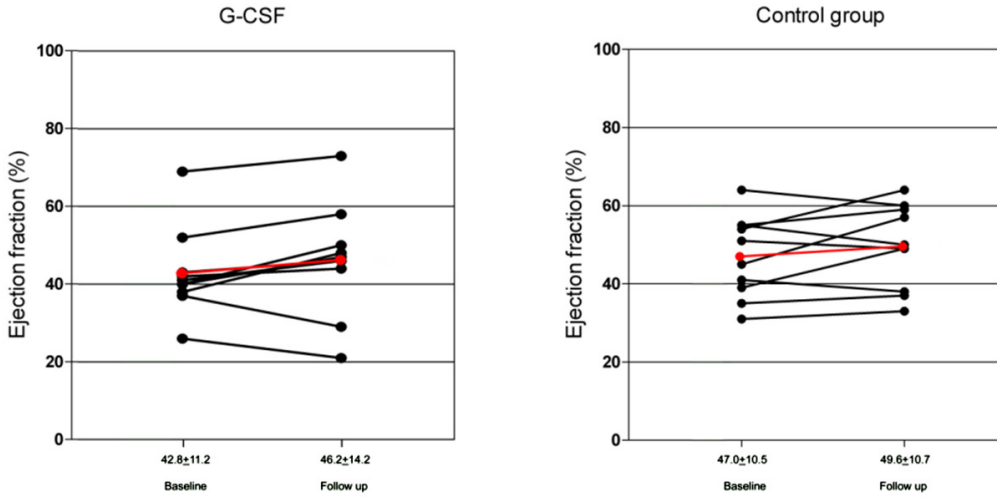

Figure 2: Changes of EF by scan in treatment and control group

tissue Doppler imaging as follows: $\frac{IVCT + IVRT}{ET}$ (IVCT= Isovolumic Contraction Time, IVRT=Isovolumic Relaxation Time, ET=Ejection Time).

The patients were seen for clinical workup and ECG in our outpatient department 1, 2, 4 and 6 months after discharge. Special attention was paid to any potential signs or symptoms of arrhythmia and angina. After 6 months, echocardiography and cardiac scan (gated SPECT) were repeated to assess the EF.

Statistical analysis

Differences in demographic characteristics between the control and the treatment groups were assessed with t tests. Each patient in both groups was used as his own control, and changes between baseline and follow-up in the control and treated groups were assessed with paired t tests. The changes from baseline to follow-up between the control and treatment groups were compared by analysis of variance with repeated measures. All the statistical analyses were performed using the SPSS version 15.0 software and P values less than 0.05 were considered as statistically significant.

Results

The results obtained from treatment and control groups are presented in this study. There were 10 patients with acute first anterior wall myocardial infarctions in each group.

The treatment group consisted of 7 (70%) men, and 3 (30%) women. Four (40%) patients in this group had hypertension, 1 (10%) had diabetes mel-

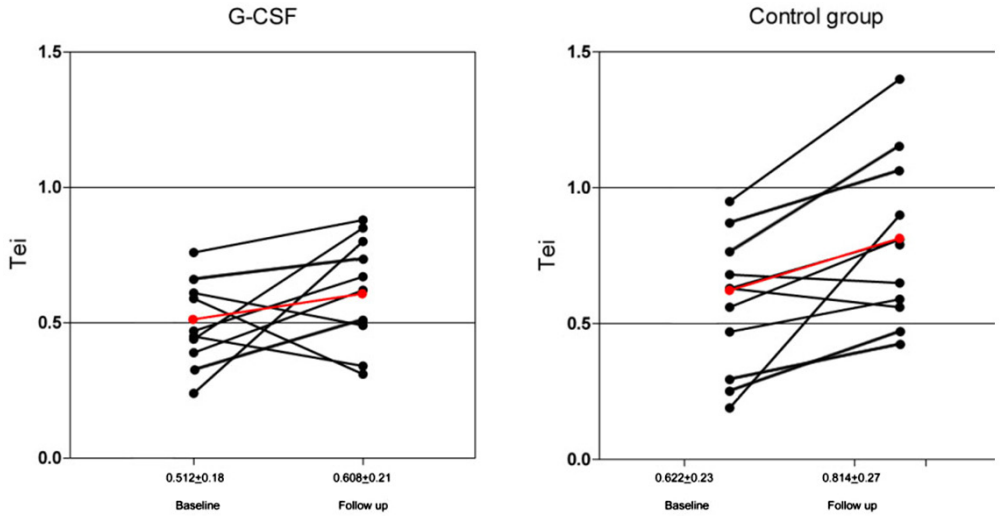

Figure 3: Changes of Tei index in control and treatment group

Table 2. Sm and Em results (cm/s)

Group		N	Minimum	Maximum	Mean	Std. Deviation
Treatment	SM1	10	3	8	6.1	1.5
	SM2	10	3	6	5.4	1.0
	EM1	10	4	8	5.8	1.6
	EM2	10	4	9	6.3	1.4
Control	SM1	10	6	10	7.4	1.3
	SM2	10	5	11	7.3	1.9
	EM1	10	5	8	7.0	1.1
	EM2	10	5	9	7.2	1.3

litus, 7 (70%) were smokers, 3 (30%) had hyperlipidemia with high LDL, 2 (20%) had a history of stable angina pectoris(IHD) and symptomatic before MI and 6 (60%) had a positive family history for ischemic heart diseases. Mean age of the treatment group was 50.6±9.2 years.

The treatment group consisted of 9 (90%) men and 1 (10%) women of which 4 (40%) had hypertension, 2 (20 %) were with diabetes mellitus, 6 (60%) were smokers, 2 (20%) had hyperlipidemia with high LDL, 4 (40%) had history of IHD and were symptomatic before MI and 3 (30%) had positive family history of ischemic heart diseases. Mean age of the control group was 51.7±15.5 year (Table 1).

Mean EF in the treatment group determined by Gated SPECT cardiac scan increased from 42.8±11.2 to 46.2±14.2, and by echocardiography elevated from 48.9 ±11.7 to 54.9±16.8, (Figures 1 and 2).

Mean ejection fraction in the control group determined by Gated SPECT cardiac scan increased from 47.0 ±10.5 to 49.6±10.5, and by echocardiography from 51.7±13.2 to 59.8±9.8. The p values for comparison of EF determined by scan (0.821) and echocardiography (0.705) were not found to be significant (Figure 2).

As shown in Table 2, data of Sm and Em obtained from echocardiography were not statistically significant between the treatment and control groups (P for Sm= 0.250 and P for Em= 0.731)

Changes of E/Em were not significant in both groups (Table 3). Changes in the Tei index was not significant in the treatment group (P=0.815); however, it was significantly deteriorated (P=0.005) in the control group (Figure 3).

Discussion

The present study describes a clinical trial of treatment with G-CSF to increase circulating bone marrow stem cells for improving myocardial function in patients after AMI. There was no significant improvement of the EF between treatment and control group (42.8± 11.2 vs. 46.2±14.2 P=0.821).However, the Tei index significantly declined in the control group, indicating a beneficial effect of G-CSF in preventing the remodeling processes in damaged myocardial tissue.

The basis of our approach was the experimental data showing improvement of cardiac function either by intracoronary injecton of mobilized bone marrow stem cells or treatment with G-CSF and stem cell factor to mobilize BMCs after myocardial infarction.[5,6,14,17] BMCs are capable of homing in the myocardium because bone marrow–derived car-

Table 3. E/Em and Tei index results

		N	Mean Rank	Sum of Ranks	P value
E\Em1 Treatment	Baseline	10	9.9	79.0	
	Follow up	10	8.2	74.0	0.541
E\Em2 Control	Baseline	10	7.9	63.5	
	Follow up	10	9.1	72.5	0.645
Tei index1 Treatment	Baseline	10	9.4	75.0	
	Follow up	10	5.3	42.5	0.815
Tei index2 Control	Baseline	10	11.7	93.5	
	Follow up	10	8.7	78.0	0.005

diomyocytes, endothelial cells, and fibroblasts were identified in the hearts of patients who had undergone sex-mismatched bone marrow transplantation or heart transplantation.[18] The partial repair of the infarcted myocardium implies a cross talk between the stem cells and the injured myocardium, and stem cell migration, proliferation, and differentiation from the border zone of the infarcted region into the zone of injury which suggested the cellular basis of improvement in cardiac function.

The population in this trial was homogenous but with some differences that could potentially influence the result such as thrombosis in myocardial infarction flow, grade before PCI, and time to PCI. This trial did not have statistical power to properly control these factors. The cardiac scan modality is especially appealing for precise and accurate measuring of ejection fraction, which is superior to other available methods such as MRI. In addition, this method allows an accurate determination of regional morphology and function. Left ventricular remodeling has been studied extensively with echocardiography and nuclear imaging.

G-CSF is a potent hematopoietic cytokine that increases the production of granulocytes and mobilizes granulocytes and stem and progenitor cells from the bone marrow into the blood circulation. Nevertheless such mobilization process is not fully understood but is mediated through enzyme release. Orlic et al.[5,6] reported favorable results after stem cell mobilization with G-CSF and stem cell factor in mice with acute myocardial infarction. A recent experiment with G-CSF after reperfused myocardial infarction in rabbits showed improvement in left ventricular ejection fraction and reduced remodeling.

Kuethe et al.[16] compared 14 patients treated with G-CSF 2 days after STEMI with 9 patients who refused G-CSF treatment. The treated group had a significantly higher increase in EF compared with the control group.

In this randomized trial, the effects of G-CSF on improvement of myocardial function in human AMI, was investigated. Results of our control group are comparable with those of recently published trials. This showed that 6 months after myocardial infarction, improvement ranging from 0.03 to 0.04 of EF, evaluated by cardiac scan (Gated SPECT method), can be expected using stent implantation for reperfusion treatment strategy compared with the EF at the time of intervention.[19] In our study, the EF evaluated by cardiac scan (Gated SPECT method) group increased by 0.05 compared with that of the control group. In our study, EF was determined before the G-CSF treatment phase about 9 to 10 days after AMI, when contribution of stunned myocardium should not have a major impact on global EF compared with the measurement at the time of intervention. Therefore, it can be assumed that the major increase in EF, from day 10 to 6 months after AMI is not a consequence of improved function of stunned areas of the myocardium. Furthermore, the improvement related to stunning is, presumably, similar in both groups. Tei index that was quantitatively evaluated by echocardiography by a fully blinded physician was deteriorated in the control group, showing a positive effect of G-CSF treatment on cardiac remodeling.

In previous studies a dose of 10 μg/kg body weight per day was suggested to result in peak CD34+ cells after 5 days of treatment. After reaching the peak of CD34+ cells, treatment was continued for about 2 days to maintain a plateau and was discontinued when CD34+ cells decreased in spite of G-CSF treatment. The mean increase of CD34+ cells was comparable with that of healthy donors, showing that processes that accompany the myocardial infarction do not influence the response of the bone marrow to G-CSF stimulation.

The time point of treatment with G-CSF was chosen 2 weeks after myocardial infarction post-PCI. (It is very important because prior studies showed that early therapy is not effective due to extensive inflammatory response and this study was performed 2 weeks post MI to abolish this effect)At this time, the acute inflammatory response, which is greatest during the first week, is completely resolved.[3] Li et al[15] and Kuethe et al.[16] showed that cardiomyocyte transplantation was not successful when performed immediately after transmural injury, compared with a more favorable outcome when it was performed later. The authors suggested that the inflammatory response is responsible for the negative effect of cell transplantation in the first days after infarction because acute inflammation is highest during early days after myocardial infarction.

Recent published data of G-CSF treatment after myocardial infarction in nonhuman primates suggested a positive effect of G-CSF on neovascularization in the paranecrotic area, but without an improvement of left ventricular function and any evidence of myocardial repair.[20] Finally, a recently published study by Kawada et al.[21] showed that mesenchymal stem cells can be mobilized by G-CSF and differentiate into cardiomyocytes in the cardiac niche after myocardial infarction in mice.

These data support the concept that stem cells can contribute to the repair of injured myocardium by being mobilized to the site of injury. These, together with the data of aforementioned studies and the findings regarding the Tei index in our study, suggest a positive effect of stem cell therapy on remodeling of myocardial tissue. However, G-CSF therapy in our study showed no significant effect on EF that was in line with previously reported findings [22,23]. The same results were also obtained in another double-blind, randomized, placebo-controlled study, using G-CSF to mobilize stem cells in MI [24]. In addition, similar results have been obtained in animal studies, showing a lack of effectiveness of stem cell therapy on cardiac function.[25]

Regarding the safety of stem cell therapy, several experimental and clinical studies have been published, which showed some unfavorable results. The major concern after stem cell transplantation is the failure of transplanted cells to integrate electrically which could theoretically lead to development of an abnormal heart beat or sudden cardiac death [26]. This problem seems to be especially associated with the transplantation of autologous skeletal myoblasts [27] but has not been observed after intracoronary application of bone marrow–derived stem cells and not in patients treated with G-CSF.[28]

The complications observed during our G-CSF therapy were fever in 3 and bone pain in 2 our patients that were controlled successfully by using paracetamol.

Taken together, these results illustrate that G-CSF treatment of patients with AMI is feasible and safe.

Granulocyte-colony stimulating factor treatment may have a favorable effect on global cardiac function but without affecting the EF under clinical conditions. Nonetheless, a randomized trial with more patients will be required to substantiate the role of this new and promising approach as an adjunctive therapy in AMI.

The most important limitation of this study was the time of performing PCI for the patients, varying from 0-7 days post- myocardial infarction, and the restricted number of patients included in the study.

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