REVIEW ARTICLE

Application of G-CSF in Congestive Heart Failure Treatment

Atena Pourtaji[1], Vajiheh Jahani[2], Seyed Mohammad Hassan Moallem[3], Asieh karimani[1] and Amir Hooshang Mohammadpour[4,5,*]

[1]*Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran;* [2]*Department of Clinical Pharmacy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran;* [3]*School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;* [4]*Department of Clinical Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran;* [5]*Pharmaceutical Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad, Iran*

Abstract: ***Introduction***: Congestive Heart Failure (CHF) is a disorder in which the heart is unable to supply enough blood for body tissues. Since heart is an adaptable organ, it overcomes this condition by going under remodeling process. Considering cardiac myocytes are capable of proliferation after MI, stimulation of neovascularization as well as their regeneration might serve as a novel target in cardiac remodeling prevention and CHF treatment. Granulocyte Colony-Stimulating Factor (G-CSF), is a hematopoietic cytokine that promotes proliferation and differentiation of neutrophils and is involved in cardiac repair after MI. So far, this is the first review to focus on GCSF as a novel treatment for heart failure.

Methods: We conducted a search of some databases such as PubMed for articles and reviews published between 2003 and 2017, with different keywords including "G-CSF", "congestive heart failure", "new therapies for CHF", "filgrastim", "*in vivo* study".

Results: GCSF exerts its beneficial effects on cardiac repair through either stem cell mobilization or direct angiogenesis promotion. All of which are capable of promoting cardiac cell repair.

Conclusion: GCSF is a promising target in CHF-therapy by means of cardiac repair and remodeling prevention through multiple mechanisms, which are effective enough to be used in clinical practice.

1. INTRODUCTION

Congestive Heart Failure (CHF) occurs when the heart is unable to pump sufficient blood through the body tissues for their needs. Its high prevalence plus its high rate of morbidity and mortality cause considerable financial and medical burden [1, 2]. As age rises, the incidence and prevalence of CHF get higher. Thereupon, it is rare under the age of 60 years [3]. Nearly 26 million adults worldwide suffer from heart failure. It is expected in developed countries that one person in five will develop heart failure during their life [4]. Heart failure has a higher mortality rate than colon, breast or prostate cancer. The incidence of sudden cardiac death is 6-9 times higher than the general population and up to 50% of

patients with CHF die suddenly. Most of these deaths are because of ventricular tachyarrhythmia [5, 6].

The heart tries to maintain the normal output level in the short term by going under a remodeling process. Although remodeling is beneficial in the short term, after a while, abnormalities may occur and finally lead to heart failure. The remodeling process has two characteristics: cardiomyocyte injury and myocardial fibrosis. Cardiomyocyte injury includes cardiomyocyte hypertrophy, necrosis and apoptosis. Myocardial fibrosis can arise either after Myocardial Infarction (MI) due to myocyte necrosis or hypertrophic cardiomyopathy, sarcoidosis, myocarditis, chronic kidney disease and toxic cardiomyopathy [1-4].

The progression of the cardiac remodeling process is based on many mediators including neurohumoral factors, cytokines, growth factors, and enzymes. The role of neurohumoral factors including angiotensin II, endothelin-1, aldosterone and norepinephrine in the progression of Left Ven-

*Address correspondence to this author at the Pharmaceutical Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad, Iran; Tel: +98-9153162909;
E-mail: MohamadpoorAH@mums.ac.ir

tricle (LV) remodeling has been well detected. Thus, an effective therapeutic approach to manage heart failure after MI is prevention of the remodeling process. In order to prevent cardiac remodeling, pharmacological agents such as ACE inhibitors and β-blockers can be used. As a result, morbidity and mortality rate is reduced. It has been recently shown that human cardiomyocytes have the ability to proliferate after MI. Therefore, it is assumed that boosting the regeneration of cardiomyocytes and stimulating of neovascularization can lead to cardiac remodeling prevention and as a result, heart failure stops progressing. Filgrastim, a Granulocyte Colony-Stimulating Factor (G-CSF), is a cytokine involved in hematopoiesis which affects the neutrophil progenitors and induces proliferation and differentiation besides its effect on immunomodulation. After MI, the infarcted region produces G-CSF. The produced G-CSF plays an essential role in cardiac repair. This beneficial role is due to either mobilizing the stem cells or direct effects such as preventing apoptosis, regulating fibrosis, improving mitochondrial function and causing angiogenesis [7].

More investigations are needed to complete our knowledge about the effectiveness of G-CSF in the management of CHF. On the other hand, there are some clinical trials that have shown the potency and effectiveness of G-CSF in the treatment of CHF patients, particularly in severe cases. Furthermore, there is some evidence of cytokine involvement in CHF pathophysiology. These studies have suggested that G-CSF administration may be an option in the treatment of CHF. We review here the effectiveness of G-CSF in CHF patients.

2. METHOD

We conducted various searches in some databases such as PubMed, Web of Science, Scopus, Science direct and clinicaltrials.gov for articles and reviews published between 2003 and 2017 with different keywords including "G-CSF", "congestive heart failure", "new therapies for CHF", "filgrastim" and "*in vivo* study". We also used the terms "mechanism" and "clinical trial". In this article about 30 related articles were used.

3. RESULTS

Recent studies have shown that many of the growth factors, cytokines and receptors are potent agents to induce cardiac repair. Angiogenesis, anti-apoptotic effects and stem cell homing are the known mechanisms of these agents to modify cardiac function and reduce LV remodeling. Mobilizing cells by using G-CSF has been suggested as a less invasive strategy for cardiac repair [8, 9].

3.1. Evidence on GCSF Beneficial Role in Heart Failure Improvement

3.1.1. Cardiac Repair After MI

GCSF preserves its healing cardiac capacity through either stem cells mobilization or angiogenesis promotion.

3.1.2. Stem Cell Mobilization

Bone Marrow Stem Cells (BMSCs) possess the capability to merge into the parenchyma of solid organs. They reach the aimed solid organ through the blood. Subsequently, they proliferate under the influence of hematopoietic growth factors such as G-CSF. As a result, the diseased organs contain plenty of stem cells. Mesenchymal Stem Cells (MSCs) are able to differentiate to endothelium cells and cardiomyocytes [1]. Thus, studies have focused on MSCs in order to find a way to improve cardiac function. It is also conceivable that G-CSF may generate an acceptable environment in the necrotic myocardium for stem cells to integrate into the tissue. This hypothesis comes from the principle that G-CSF can change the expression of a chemokine which is involved in shifting the BMSCs from the blood to the injured myocardium, called stromal cell-derived factor-1 [9, 10].

In addition, G-CSF influences the Akt/endothelial Nitric Oxide (NO) synthase pathway. As a result, the production of NO increases. The produced NO makes the endothelial cells to proliferate and migrate [9].

As well as mobilizing BMSCs to the necrotic myocardium, novel evidence suggests that G-CSF affects the myocardium directly and protects the myocardiocytes. Thereupon, it is conceivable that G-CSF changes the damaged myocardium to a milieu that is more appropriate for stem cells to merge and to engraft.

Neutrophils and macrophages speed up the healing process. At the first step, the cardiac healing regulation depends on the infiltration of inflammatory cells that is mediated by G-CSF. G-CSF calls up the monocytes/macrophages that possess the ability to turn into myofibroblast and cardiac repair enhances subsequently. Using G-CSF enhanced the expression of transforming growth factor-1 and improved the reparative collagen synthesis in the infarcted region. Therefore, G-CSF promoted the early post-infarct expansion of the ventricles [11, 12].

In any situation that subcutaneous G-CSF was injected with its easy route of administration, G-CSF was a noteworthy therapeutic option leading to cardiac repair and probably regeneration of the injured myocardium due to delayed reperfusion therapy.

3.1.3. Angiogenesis Promotion

As mentioned above, G-CSF induces the endothelial and vascular smooth muscle cells to proliferate and migrate. Therefore, this effect may afford development of collateral arteries. As the collateral artery grows, the coronary blood flow will improve. This principle indicates that reactive oxygen species that are produced under the influence of G-CSF therapy in vitro, may play a possible role in the secretion of angiogenic factors [9, 13]. Hence, mobilizing the stem cells by G-CSF bring about angiogenesis that protects the heart from ischemia.

3.1.4. GCSF Signaling Pathways Effect on Cell Survival and Proliferation

G-CSF may act as an anti-apoptotic agent for cardiac myocytes due to its effect on activating Akt/ protein kinase B pathway. This pathway is known by its cell protection and cell survival effect. In addition, G-CSF mobilizes the CD34+ cells which initiate neoangiogenesis in the infarcted heart. As a result, inhibition of apoptosis of cardiomyocytes and reduction of collagen deposition and scar formation occur.

Cardiomyocytes and endothelial cells express G-CSF receptor (G-CSFR). The downstream signaling pathways including Janus-activated Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) are critical for G-CSF to function in the heart [14]. As the cells proliferate, ligand binding is followed by homodimerization of G-CSFR and activation of associated JAK tyrosine kinase. After the JAK pathway is activated, G-CSFR is phosphorylated and STAT transcription factors are activated. The activated transcription factors move to the nucleus and prompt gene transcription. STAT3 is a protective factor for the heart. It prevents pathophysiologic stresses such as ischemia, mechanical stresses and cytotoxic agents from harming the heart. In addition, activation of G-CSFR is followed by induction of tyrosine phosphorylation of SH2- containing protein Shc. The phosphorylated Shc activates the Ras pathway and Mitogen-Activated Protein (MAP) kinases and induces immediate early genes. In non-proliferating terminally differentiated cells, G-SCF influences other pathways that have no apparent connection to the well-known Jak-STAT pathway or the Ras-MAP kinase pathway [7, 9, 10].

3.1.5. Effect on LV Remodeling and Cardiac Output

Cardiac remodeling is a compensatory process to preserve the normal heart function. However, it gently turns into a vicious circle and results in CHF.

G-CSF affects the expression of molecules that are known to result in adverse cardiac remodeling (particularly scar formation), such as angiotensin II type 1 receptor, transforming growth factor-β1 and tumor necrosis factor-α [10]. In addition, G-CSF possesses pleiotropic effects that can improve impaired mitochondrial electron transport and oxygen consumption in the cardiac tissue [9]. Recent animal studies have shown G-CSF can improve ejection fraction that prevents LV remodeling [9, 10, 15].

Furthermore, it has been reported that failing post-MI hearts downregulate the expression of GATA-4. GATA-4 is a transcription-related protein that regulates the expression of sarcomeric proteins in the cardiac myocytes. Applying G-CSF in post-MI hearts results in a restored GATA-4 expression that hypertrophies the remaining cardiac myocytes and attenuates fibrosis [16].

Although the currently believed concept that cardiomyocytes do not possess the ability to regenerate, it has been lately reported that post-MI human cardiomyocytes proliferate. Hence, it is assumed that boosting the regeneration of cardiomyocytes with the combination of inducing neovascularization may prevent the heart from remodeling and progressing to heart failure. This hypothesis can be achieved through G-CSF therapy [7].

Furthermore, applying G-CSF can increase the density of arterial vessels in the margin of the infarct. It has been also reported that using G-CSF can enhance cardiac output in an experimental mice model of MI. Similar to these findings, it was shown in a non-ischemic rabbit model of heart failure that G-CSF decelerated the progression of LV dysfunction. Echocardiography was the modality to detect LV dysfunction. Possibly, this effect is due to the increment of arterial and capillary density and also connexin 43 expression in injured myocardium. In addition, increased connexin 43 expression may be responsible for preventing cardiac arrhythmias.

Prolonged action potential duration and increased spatial and temporal propagation of repolarization can be results of chronic G-CSF therapy. Therefore, polymorphic ventricular tachyarrhythmia may occur more frequently due to triggered activity. This effect is in contrast to the increased expression of connexin 43 and its preventing effect on arrhythmia [5, 10].

4. PRECLINICAL AND CLINICAL STUDIES EVALUATING G-CSF EFFECTIVENESS IN CHF

4.1. Preclinical Evidence on Adding G-CSF in CHF

4.1.1. Animal Studies

Recent studies have reported the G-CSF effect on heart failure. In consideration of studying the G-CSF effect on cardiomyocyte's mitochondria, a study was conducted on eight-week-old male mice in which 5 mg/kg/day doxorubicin was injected 6 times for 2 weeks followed by administration of 100 µg/kg/day G-CSF for 5 continued days. Therapeutic doses of doxorubicin induce immutable dilated cardiomyopathy accompanied by myocardial necrosis and severe pump failure. Although injecting mild doses of doxorubicin in vivo resulted in disorganization of mitochondria in the cardiomyocytes and diastolic dysfunction, but the typical cardiomyopathy characteristics did not appear. This study indicated that the improvement of hemodynamic and mitochondrial function of the heart can be achieved by administrating G-CSF particularly in the early phase of cardiac damage. Furthermore, cellular respiration was improved in the treated group. Cellular respiration is a factor to assess the fate of cardiomyocytes [17].

Another study was performed on 24 rabbits suffering from heart failure. Rabbits received G-CSF for 17±4 days. G-CSF therapy affected arteriogenesis and angiogenesis leading to improvement of myocardial contractility and slowed down the development of LV dysfunction in the rabbits with non-ischemic heart failure. Disappointingly, long-term G-CSF treatment led to an increased occurrence of ventricular tachyarrhythmia. Hence, choosing cytokines in the treatment of heart failure requires accurate cardiac monitoring with the aim of prevention of myocardial repolarization [5].

In another study investigators aimed to show the efficacy of G-CSF in a high-fat diet (HFD)-induced cardiovascular diseases associated with obesity and type 2 diabetes mellitus. They enrolled twenty-week old male mice and induced obesity by feeding them with HFD over thirty-six weeks. After the feeding period, rabbits suffered from considerable cardiac injuries such as fibrosis and reduced cardio-pulmonary capacity. Henceforth, the rabbits underwent G-CSF therapy with a daily dose of 200 µg/Kg/day for five days. This protocol was repeated for three times with an intervening time of seven days. After the G-CSF therapy, they observed several beneficial effects such as reduced cardiac fibrosis, decreased insulin levels, enhanced cardiopulmonary capacity, accelerated body weight reduction and reversed cardiac changes. Therefore, it seems that G-CSF is a protective agent for car-

diomyocytes to prevent hypertrophy besides its ability to reverse diabetic cardiomyopathy [15].

In another effort to find the effect of G-CSF on protecting heart failure, MI-induced mice were divided into three different groups to apply three different protocols of G-CSF administration. The first protocol included administration of G-CSF with smaller doses and longer intervening time. MI was induced in forty-six mice and after twelve weeks of MI, twenty-four mice survived (survival rate=52%). They divided the surviving mice into two groups after examining echocardiographic parameters. One group received subcutaneous recombinant G-CSF for four consecutive weeks with a dose of 10 mg/Kg/day on the first five days of each week. The other group was administered with an equal volume of saline. The second protocol was done to assess the delayed effects of G-CSF after a long-term G-CSF therapy even after stopping the treatment. They induced MI in thirty-six mice of which twenty mice survived after eight weeks (survival rate=56%). They examined the echocardiographic parameters and separated the surviving mice into two groups (n=10 each). With the same method mentioned in Protocol-1, G-CSF and saline were injected over two weeks.

The third protocol focused to evaluate the effect of G-CSF in the induction of myocardial regeneration by bone marrow cells. MI was created in fifteen mice. Twelve weeks later, nine mice survived and G-CSF (n=5) or saline (n=4) was administered. The therapy period was four weeks with the same protocol mentioned above.

After sixteen weeks of MI, the results showed G-CSF stimulated expression of G-CSFR in the injured myocardium with a positive feedback depending on itself (autoinduction). It is known that the infarcted murine hearts express G-CSF endogenously. G-CSF therapy reduced heart fibrosis and hypertrophied surviving cardiomyocytes.

Another finding of this study was the existence of bone marrow-derived cells in hearts with old MI that contained small numbers of fibroblast-like cells and leukocytes in the absence of cardiac myocytes and vascular cells. It was concluded that G-CSF was not the reason for their presence. This disappointing finding indicates that G-CSF contributes slightly to regenerate myocardium after an old MI. Nevertheless, ruling out the possibility that a number of bone marrow-derived cardiac myocytes were present before is not possible. This is because the entire heart was not examined. It is notable that when administrating G-CSF, its effects lasted on heart function for at least two weeks [16].

Another study was performed to show the effects of G-CSF on morphological and physiological recoveries in the injured hearts. Heart injury was created by isoproterenol, a cardiotoxic agent, with a daily dose of 5/mg/Kg/day over seven consecutive days. Heart injury was specified by hypertrophy of ventricles, cardiac fibrosis and increased mean arterial pressure and pulse rate. G-CSF healed the cardiac injury probably with the mechanism of shifting the cells from the bone marrow to the blood that some of them merged to the damaged heart. G-CSF therapy led to regression of cardiac fibrosis in the absence of attenuating hypertrophy or improving hemodynamic parameters. This study showed that frequent β-adrenergic stimulation is the mechanism of G-CSG in partially healing LV remodeling.

With the purpose of finding the role of mobilized cells in cardiac repair, mobilized stem cells from one group of healthy mice were taken and transferred into another group that suffered from heart injury. The results showed the same outcome as the G-CSF. In addition, inoculation restored the arterial pressure faster. Impressively, the myocardium presented with some of the transferred cells and also a portion of them had a positive αSMA marker. These results show that the cells with no dependency to G-CSF are responsible for restitution of the damaged heart [18].

Another study was conducted on forty-five male rats poisoned with CO for sixty minutes at a dose of 3000 ppm for cardiac ischemia induction. Following the poisoning process, the rats instantly underwent G-CSF therapy with a dose of 100 mg/Kg/day for five continuous days. In order to find whether proteins are expressed after receiving G-CSF, they performed western blot analysis. These proteins included JAK2, p-JAK2, STAT3, p-STAT3, Akt1 and p-Akt1. G-CSF protects the cardiac myocytes after ischemia/reperfusion by Akt1 phosphorylation [13].

Exposure to carbon monoxide (CO) is followed by apoptosis of rat cardiac myocytes. The results indicated that the involvement of JAK2/STAT3 and PI3k/Akt in preventing apoptosis in rat cardiac myocytes is evident. Analyzing the Western blot results showed that G-CSF increased the phosphorylated form of Akt1, JAK2 and STAT 3 without changing the total amount of the mentioned proteins. In other words, G-CSF therapy led to cardiac protection due to its effect on increasing phosphorylated proteins. In addition, G-CSF reduces transcription of apoptosis-inducing proteins such as Bad and caspase 3 [19] It is expected that after MI about one-half of all patients develop CHF and is characteristic for acute changes in LV function [20]. Accordingly, G-CSF can improve the function of a chronic failed heart. The summary of human and animal studies is presented in Table **1**.

4.2. Human Study

After animal studies showed that the heart is not a postmitotic organ but can be regenerated from cardiac and noncardiac cells, several clinical trials have been carried out that aimed to obtain myocardial repair in patients with ischemic CHF.

4.2.1. Cases of Severe CHF with Cardioverter Implantable Defibrillators (ICDs)

A study was conducted that enrolled 6 cases of severe heart failure with left ventricular ejection fraction less than 35 percent who had cardioverter implantable defibrillators (ICDs) in situ. Their therapeutic plan was the administration of G-CSF with escalating doses for safety assurance. Patients underwent paraclinical assessments including electrocardiography studies, plasma cytokine, and flow cytometry for finding CD34+ cell count before G-CSF therapy. Drug administration was halted in 6 patients because of elevations in alkaline phosphatase. Among these patients, 3 used a whole dose of 25 g/kg and 2 received 22.5 g/kg for a period of five

Table 1.   Summary of human and animal studies [5, 13, 15-18, 21, 22, 25, 27].

Drug	Dose and Duration	Study Type	Study Sample	Assessment or Clinical Response
Filgrastim (G-CSF)	100 µg/kg/day of G-CSF for five consecutive days	Animal study	Male 8-week year old mice	Improved hemodynamic cardiac function, mitochondrial respiration and cellular mitochondrial function in the early phase of cardiac injury. It also prevents Dox-induced drop in mitochondrial membrane potential [17]
Filgrastim (G-CSF)	10 µg/kg G-CSF for over 17 ± 4 days	Animal study	24 female rabbits induced heart failure	Slower progression of echocardiographically detected LV dysfunction. Chronic G-CSF therapy results in the facilitated ventricular tachyarrhythmias occurrence [5].
Filgrastim (G-CSF)	Three courses of a daily injection (200 µg/kg/day in saline, intraperitoneally) for 5 days, including a 7-day interval between each course.	Animal study	6 Twenty-week year mice	G-CSF can Prevent cardiac hypertrophy [15]
Filgrastim (G-CSF)	10 mg/kg/day on the first 5 days of each week, continued for 4 weeks	Animal study	24 survived mice after induced MI at 24 weeks age	Hypertrophy in surviving cardiomyocytes and reduced myocardial fibrosis [16]
	20 survived mice after induced MI		(n¼10) for 2 weeks using the same method described in Protocol-1	
	9 of the surviving mice 12 weeks post-MI		(n¼5) or solvent (n¼4) for 4 weeks using the same method as described in Protocol-1	
Filgrastim (G-CSF)	4 co-administrations of isoproterenol and GCSF (300 µg kg–1 day–1, 4 days, s.c.)	Animal study	Mice treated with daily administrations of isoproterenol 5 mg/kg/day, 7 days	Promoted regression of fibrosis without diminishing hypertrophy or hemodynamic parameters [18]
Mobilized bone marrow-derived cells	24 h after the last administration of isoproterenol			
	30 days after the last administration of isoproterenol			
Filgrastim (G-CSF)	100 mg/kg/ day subcutaneously for five consecutive days	Animal study	Forty-five male rats poisoned with CO for cardiac ischemia induction	G-CSF protects the cardiac myocytes after ischemia/reperfusion by Akt1 phosphorylation [13]
Filgrastim (G-CSF)	Total dose of 22.5 (n=2) to 25 g/kg (n=3) within 5 days, and 1 patient received a total dose of 10 g/kg	Human study	6 patients with advanced heart failure, left ventricular ejection fraction <35%, and implantable defibrillators in situ	Mobilization of hematopoietic stem cells in advanced heart failure and improved left ventricular function in the ischemic subset of patients [21]
Filgrastim (G-CSF)	10 µg/kg/day for 5 days	Human study	30 patients with previous MI and an ischemic heart failure in NYHA and/or CCS classes ≥3 unsuitable for surgical or percutaneous revascularization	Improved angina stability, treatment satisfaction and quality of life [22]
Filgrastim (G-CSF)	Starting dose: 480 µg Sc bid) adjusted daily to reach a high level of stem cell mobilization, four 10-day treatment periods interrupted by treatment-free intervals of equal length) until 70 days	Human study	16 male patients with chronic heart failure due to dilated (DCM; n = 7) or ischemic cardiomyopathy (ICM; n = 9)	Possibly effective in improving physical performance in patients with CHF [25]
Filgrastim (G-CSF)	(10 µg/kg/day) for 5 days	Human study	60 patients with non-ischemic DCM	Improvement in cardiac function accompanied by improvement in symptoms [27]
	Bone marrow harvest after 5 days of G-CSF and received IC infusion of autologous BMC			
	Bone marrow harvest after 5 days of G-CSF but received IC infusion of serum only			

days. Another remained patient received a total dose of 10 g/kg before stopping the drug. The dose was increased if the patients showed no effect of drug with the first dose administration. However, all patients responded to the initial doses and dose adjustment was not required. Patients follow up with laboratory and clinical assessments was conducted at 6, 8, and 10 days after drug administration and then in 6 weeks, three months, and nine months. A rise in the level of CD34+ cells in the peripheral blood more than 10 cells/µl was the primary endpoint. This number of CD34+ cells is acceptable for transplantation. The secondary endpoints were safety, Left Ventricular Ejection Fraction (LVEF) changes and altered levels of cytokine in the plasma. Administration of a dose of 5 µg/kg/day of G-CSF for a period of five days increased the amount of CD34+ hematopoietic stem cells. They found notable improvement in left ventricular ejection fraction in 4 patients who suffered from ischemic cardiomyopathy after a 9-month follow up. Also, a non-statically significant decrease was found in left ventricular end-systolic and end-diastolic dimensions. Plasma cytokine (interferon-γ, TNF-α, or IL-2, -4, -5) levels did not improve significantly during the administration of G-CSF. However, plasma IL-10 level increased notably. After 6 weeks, it returned to the baseline level. The patients were followed-up for 9 months and cardiovascular adverse events did not occur in any of the patients. Finally, it has been proposed that low-dose G-CSF therapy can significantly mobilize hematopoietic stem cells in patients with advanced heart failure. Also, it can improve left ventricle function in patients with ischemic cardiomyopathy. All these benefits accompany little side effects of G-CSF. Mobilizing bone marrow-derived hematopoietic stem cells is a possible treatment option for acute MI and CHF. Neutrophilia was seen with administration of G-CSF, however, this did not result in discontinuing G-CSF treatment in any patient. HF patients have lower plasma levels of IL-10. The lower the IL-10 serum level is detected; the more severe HF we expect. This is due to inhibition of release of TNF-α by IL-10 from mononuclear cells in the peripheral blood of patients suffering from CHF. G-CSF influences the production of IL-10 and as a result, decreases *ex-vivo* production of TNF-α. Therefore, it is possible that besides the effect of G-CSF on neovascularization and stem cell trans-differentiation into myocardial cells, G-CSF changes the overall effect of cytokines to anti-inflammatory phenotype. Thus, this study on a small population of patients with advanced heart failure shows that G-CSF therapy can result in further complementary benefits for cardiac myocardium besides benefits of hematopoietic stem cell transplantation [21]. The limitation of this study was the small study population as more reliable findings can be achieved by studies on larger populations [21].

4.2.2. Cases of Severe CHF with *a* History of MI

Another study enrolled 13 patients with a history of MI and ischemic heart failure with a severity of New York Heart Association (NYHA) and/or Canadian Cardiovascular Society (CCS) class ≥3. The selected patients were un-appropriated for revascularization and also their symptoms did not change after receiving one month of medical treatment or implantation of a biventricular pacemaker. Patients underwent clinical examinations including assessment of

stress/rest gated SPECT imaging, and indexes of symptoms and quality of life at the beginning of the study and 4 months after the treatment. NYHA and CCS classes, the Seattle Angina Questionnaire (SAQ), the Minnesota Living with Heart Failure Questionnaire (MLHFQ) and a Visual Analogue Scale (VAS) were indexes in order to evaluate symptoms and quality of life. Briefly, the SAQ is a 19-item questionnaire that assesses the effect of angina on physical activity, recent severity changes of angina, angina attack frequency, treatment satisfaction and disease perception. This questionnaire has a range from 0 to 100; the higher score the patients earns; the better health status is expected. The MLHFQ evaluates how HF and its treatment affect a patient's quality of life. This questionnaire contains 21 facets about how HF has prevented the patients from living as they desired and asks them to score these facets from 0 to 5. The summation of answers to these 21 questions is the global score. 99mTechnetium-sestamibi gated-SPECT was performed to asses LV volumes, perfusion and function. These indexes were measured according to Cedars-Sinai's criteria. In order to evaluate the number of CD34+ cells, a blood sample was collected at the beginning of the study and at day 5 and 10 after administrating G-CSF. NYHA and CSS classes showed significant changes that they improved from 3 (IR 2.5-3) to 2 (IR 1-2.5) and from 3 (IR 1-3) to 1 (IR 1-2), respectively. NYHA and CSS classes improved more than one in eight and five patients, respectively. Furthermore, physical limitation, frequency and stability of angina, quality of life and perfusion scores showed significant improvement. It is improbable the mobilized stem/progenitor cells from the bone marrow engraft to the cardiac tissue. In addition, it seems that G-CSF has no effect on the myocyte apoptotic pathway and as a result, undesirable remodeling is not prevented. According to this article using G-CSF is safe in patients suffering from ischemic heart failure and/or persistent angina. Ischemic heart failure cases that are not a candidate for revascularization, respond better to G-CSF possibly due to reduced stress-induced ischemia [22]. The reduced stress-induced ischemia is probably responsible for the betterment of symptoms and quality of life. Despite the improvement of symptoms and quality of life, no significant change in LV ejection fraction was detected, that was the primary endpoint in most of the clinical trials on cytotherapy in acute MI and CHF. This is because of poor correlation between ejection fraction and symptoms such as angina and breath shortness and consequently to the quality of life [23]. However, it is difficult to find a clear correlation among myocardial perfusion, quality of life and symptoms. This study showed a considerable improvement in stress myocardial perfusion. This finding is a possible explanation for symptom improvement and better quality of life in the patients. Due to the small sample size, efforts to find the predictors for good response to treatment failure. Thus, without any other effective treatment options, in patients that are not an appropriate candidate for revascularization and have severe symptoms on optimal medical treatment, using G-CSF can be a proper option [22]. This study is mainly limited by its observational nature. In order to show the long-term beneficial effects of a novel treatment, it is better to design a randomized double-blind clinical trial [24].

4.2.3. Cases of Severe CHF with Dilated (DCM) or Ischemic Cardiomyopathy (ICM)

In another clinical trial, sixteen male patients who suffered from CHF, NYHA functional class III or IV owing to dilated (DCM; n=7) or ischemic cardiomyopathy (ICM; n=9) participated. The patients received four courses of G-CSF therapy subcutaneously. Each course was 10 days' duration and was separated from the next course with a 10-day treatment-free interval. Control visits were performed monthly for 6 months after starting the treatment. The dose of G-CSF (starting dose: 480 μg sc bid) was adjusted on a daily basis to reach a leukocyte count of 45,000/μl by day 4 and maintain a level of 45,000 – 50,000/μl until day 10. This target range was chosen because it had previously been shown to be well tolerated and associated with a high level of stem cell mobilization. Leukocyte counts were obtained daily, and CD34+cells were determined on weekdays by flow-cytometry. Safety and efficacy analyses were done on day 1 and day 10 of each treatment cycle and then after monthly until day 180. Safety analyses included physical examination, ultrasonography of the spleen, electrocardiogram and laboratory investigations. In order to assess efficacy, echocardiography, six-minute walk test and evaluation of NYHA classification were performed. The patients were followed up monthly until six months after starting the treatment [25]. The peak CD34+ cell counts were unchanged in every course. Following four G-CSF administration, nine (4 DCM, 5 ICM) out of twelve patients showed considerable improvement in six-minute walking distance and NYHA class. Opposite to these findings, none of 8 ICM controls had a change in NYHA class during a similar time period. Echocardiographic parameters did not change significantly during the study. Surprisingly, the response in the DCM group was equal to the ICM group. The greatest concern was the occurrence of ventricular fibrillation and impermanently increased angina with administrating G-CSF. The study may reveal that patients with coronary artery disease receiving G-CSF can be at a major risk. Due to structural reasons, patients with DCM are at a lower risk of high leukocyte count adverse effects compared to patients with ICM [25]. Mobilizing the stem cells consecutively by using G-CSF in the mentioned study was practicable. Also, it seems that it improves physical performance in patients suffering from CHF. Improving physical performance by administrating G-CSF has to be compared with its possible hazards in patients suffering from ICM [22, 25, 26]. The most notable limitation of this study was the small sample size and in addition, the relation between symptom improvement and placebo effect could not be excluded due to the absence of a control group. Consequently, a placebo-controlled, randomized, blind trial with a larger scale on DCM patients is needed.

A phase II research has been accomplished to assess the efficacy of G-CSF monotherapy or in combination with BMC as an IC autologous therapy in DCM patients. To assess the cardiac function, a symptom evaluation, exercise test, and heart failure biomarker evaluation were conducted. The patients that were included in the study were non-ischemic DCM cases with ejection fraction under 45% and New York Heart Association (NYHA) ≥ 2. The study groups were categorized in 4 groups including peripheral subcutaneous injection as 'peripheral placebo group', subcutaneous

G-CSF injection for a 5-day period as 'peripheral G-CSF group', infusion of BMC as an IC autologous therapy after G-CSF injection in a 5-day period and bone marrow aspiration as 'IC BMC group', and IC infusion of serum after G-CSF injection in a 5-day period and bone marrow aspiration as 'IC serum group'. In order to distribute the bone marrow cells equally in the epicardial vessels, stop flow method was used. The primary results in global LVEF changes and myocardial mass LV volumes and were assessed at the end of 3 months and 1 year of follow up. Patients underwent cardiac Computed Tomography (CT) or Cardiovascular Magnetic Resonance (CMR) at baseline and 3 months of follow up. Furthermore, in secondary endpoint, changes in NT-proBNP level, VO2 peak to evaluate exercise capacity, quality of life assessment with European Quality of Life-5 Dimensions (EQ5D) and NYHA class, and Kansas City Cardiomyopathy Questionnaire (KCCQ) were conducted at 3 months and 1 year follow up compared to baseline. The results of following up the patients every three months revealed that LV ejection fraction enhanced in the group receiving both G-CSF and MNC therapy at 3 months, which was maintained at 1 year. In addition, LVEF improvement was related to the considerable improvement of quality of life, exercise capacity and NYHA classification and N-terminal pro-brain natriuretic peptide decreased in this group. However, LV ejection fraction or any of these endpoints did not change considerably in the other treatment groups at either 3 months or 1 year [27]. LVEF improvement was related to the considerable improvement of quality of life, exercise capacity and NYHA classification. This study was limited by its small study population and not being completely blinded across all groups because of the invasive procedure of bone marrow harvest. Although the trial was small in scale, it met its statistical endpoint criteria. It is possible that due to the small study population, the trial failed to show notable changes in LVESV and LVEDV.

Despite the small sample size in all groups, the results of this study are fairly persuasive. Briefly, this study contributes to the existent knowledge that stem cell therapy is effective in patients suffering from non-ischemic DCM and also possibly ischemic DCM. Although the aura of ambiguity in this field, this clinical trial suggests consideration of G-CSF therapy in combination with MNCs or other cell lines in the treatment of heart diseases [27].

These results are in accord with three other studies that administered CD34+ cells mobilized by G-CSF into the coronary arteries or endocardium. These three small non-controlled studies enrolled patients suffering from non-ischemic DCM. Transplanting CD34+ cells into the endocardium led to a better improvement of ventricular function, the more reduced serum level of NT-proBNP and greater exercise capacity compared with the intracoronary administration. Furthermore, after following up the intracoronary group for 5 years, improvement of the remaining myocardial perfusion and 6-min walking distance was seen [27-30].

CONCLUSION

In summary, after reports which state that G-CSF mobilizes bone marrow stem cells (BMSCs) into infarcted hearts and accelerates the differentiation of vascular cells and car-

diac myocytes, investigating the use of G-CSF in the treatment of heart disease has attracted researcher's attention. G-CSF through different mechanisms including prevention of left ventricular remodeling and dysfunction after MI, by a decrease in apoptotic cells and an increase in vascular cells, angiogenesis promotion and activation of various signaling pathways such as Akt, extracellular signal-regulated kinase, and Janus kinase 2/signal transducer and activator of transcription 3. These findings suggest that G-CSF not only induces mobilization of stem cells and progenitor cells but also acts directly on cardiomyocytes. Therefore, G-CSF may be utilized as a novel agent to have protective and regenerative effects on the injured myocardium. It will be necessary to confirm the safety and efficacy of its administration by more clinical trial, but our findings suggest G-CSF administration may represent a new therapeutic strategy for treating patients with chronic heart failure.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

REFERENCES

[1]   Liu T, Song D, Dong J, *et al*. Current understanding of the pathophysiology of myocardial fibrosis and its quantitative assessment in heart failure. Front Physiol 2017; 8: 238.

[2]   Lucas C, van Pol P, Smeets JE, Niesing M, Verwey H, Beeres S. Heart failure in 2015: Let's get organised! Netherlands Heart J 2015; 23(9): 447-9.

[3]   van Riet EE, Hoes AW, Wagenaar KP, Limburg A, Landman MA, Rutten FH. Epidemiology of heart failure: the prevalence of heart failure and ventricular dysfunction in older adults over time. A systematic review. Eur J Heart Fail 2016; 18(3): 242-52.

[4]   Ponikowski P, Anker SD, AlHabib KF, *et al*. Heart failure: Preventing disease and death worldwide. ESC Heart Fail 2014; 1(1): 4-25.

[5]   Milberg P, Klocke R, Frommeyer G, *et al*. G-CSF therapy reduces myocardial repolarization reserve in the presence of increased arteriogenesis, angiogenesis and connexin 43 expression in an experimental model of pacing-induced heart failure. Basic Res Cardiol 2011; 106(6): 995-1008.

[6]   Alexander RW, Schlant RC, Fuster V. Hurst's the heart, arteries and veins: McGraw-Hill, Health Professions Division; 1998.

[7]   Takano H, Qin Y, Hasegawa H, *et al*. Effects of G-CSF on left ventricular remodeling and heart failure after acute myocardial infarction. J Mol Med 2006; 84(3): 185-93.

[8]   Orlic D, Kajstura J, Chimenti S, *et al*. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 2001; 98(18): 10344-9.

[9]   Nagai T, Komuro I. Gene and cytokine therapy for heart failure: Molecular mechanisms in the improvement of cardiac function. Am J Physiol Heart Circ Physiol 2012; 303(5): H501-H12.

[10]   Kurdi M, Booz GW. G-CSF-based stem cell therapy for the heart—unresolved issues part A: Paracrine actions, mobilization, and delivery. Congest Heart Fail 2007; 13(4): 221-7.

[11]   Fujita J, Mori M, Kawada H, *et al*. Administration of granulocyte colony-stimulating factor after myocardial infarction enhances the recruitment of hematopoietic stem cell-derived myofibroblasts and contributes to cardiac repair. Stem Cells 2007; 25(11): 2750-9.

[12]   Ping P, Anzai T, Gao M, Hammond H. Adenylyl cyclase and G protein receptor kinase expression during development of heart failure. Am J Physiol Heart Circ Physiol 1997; 273(2): H707-H17.

[13]   Hasegawa H, Takano H, Iwanaga K, *et al*. Cardioprotective effects of granulocyte colony-stimulating factor in swine with chronic myocardial ischemia. J Am Coll Cardiol 2006; 47(4): 842-9.

[14]   Freeman K, Lerman I, Kranias EG, *et al*. Alterations in cardiac adrenergic signaling and calcium cycling differentially affect the progression of cardiomyopathy. J Clin Invest 2001; 107(8): 967.

[15]   Daltro PS, Alves PS, Castro MF, *et al*. Administration of granulocyte-colony stimulating factor accompanied with a balanced diet improves cardiac function alterations induced by high fat diet in mice. BMC Cardiovasc Disord 2015; 15(1): 162.

[16]   Li Y, Takemura G, Okada H, *et al*. Treatment with granulocyte colony-stimulating factor ameliorates chronic heart failure. Lab Invest 2006; 86(1): 32.

[17]   Hiraumi Y, Iwai-Kanai E, Baba S, *et al*. Granulocyte colony-stimulating factor protects cardiac mitochondria in the early phase of cardiac injury. Am J Physiol Heart Circ Physiol 2009; 296(3): H823-H32.

[18]   Nieto-Lima B, Cano-Martinez A, Zarco-Olvera G, Masso-Rojas F, Paez-Arenas A, Guarner-Lans V. GCSF partially repairs heart damage induced by repetitive beta-adrenergic stimulation in mice: Potential role of the mobilized bone marrow-derived cells. Int J Pharmacol 2016; 12(7): 689-700.

[19]   Hashemzaei M, Imen Shahidi M, Moallem SA, Abnous K, Ghorbani M, Mohamadpour AH. Modulation of JAK2, STAT3 and Akt1 proteins by granulocyte colony stimulating factor following carbon monoxide poisoning in male rat. Drug Chem Toxicol 2016; 39(4): 375-9.

[20]   Wolk MJ, Scheidt S, Killip T. Heart failure complicating acute myocardial infarction. Circulation 1972; 45(5): 1125-38.

[21]   Joseph J, Rimawi A, Mehta P, *et al*. Safety and effectiveness of granulocyte-colony stimulating factor in mobilizing stem cells and improving cytokine profile in advanced chronic heart failure. Am J Cardiol 2006; 97(5): 681-4.

[22]   Leone AM, Giannico MB, Bruno I, *et al*. Safety and efficacy of G-CSF in patients with ischemic heart failure: The CORNER (Cell Option for Recovery in the Non-Eligible patients for Revascularization) study. Int J Cardiol 2011; 150(1): 75-8.

[23]   Wenger NK. Quality of life: Can it and should it be assessed in patients with heart failure? Cardiology 1989; 76(5): 391-8.

[24]   Rana JS, Mannam A, Donnell-Fink L, Gervino EV, Sellke FW, Laham RJ. Longevity of the placebo effect in the therapeutic angiogenesis and laser myocardial revascularization trials in patients with coronary heart disease. Am J Cardiol 2005; 95(12): 1456-9.

[25]   Hüttmann A, Dührsen U, Stypmann J, *et al*. Granulocyte colony–stimulating factor–induced blood stem cell mobilisation in patients with chronic heart failure. Basic Res Cardiol 2006; 101(1): 78-86.

[26]   Matsubara H. Risk to the coronary arteries of intracoronary stem cell infusion and G-CSF cytokine therapy. Lancet 2004; 363(9411): 746-7.

[27]   Hamshere S, Arnous S, Choudhury T, *et al*. Randomized trial of combination cytokine and adult autologous bone marrow progenitor cell administration in patients with non-ischaemic dilated cardiomyopathy: The REGENERATE-DCM clinical trial. Eur Heart J 2015; 36(44): 3061-9.

[28]   Kastrup J. Revival of cytokine therapy in heart failure? Eur Heart J 2015; 36(44): 3070-3.

[29]   Vrtovec B, Poglajen G, Lezaic L, *et al*. Comparison of transendocardial and intracoronary CD34+ cell transplantation in patients with nonischemic dilated cardiomyopathy. Circulation 2013; 128(11 Suppl 1): S42-S9.

[30]   Lezaic L, Socan A, Poglajen G, *et al*. Intracoronary transplantation of CD34+ cells is associated with improved myocardial perfusion in patients with nonischemic dilated cardiomyopathy. J Cardiac Fail 2015; 21(2): 145-52.