J Mol Med (2006) 84: 185–193
DOI 10.1007/s00109-005-0035-z

REVIEW

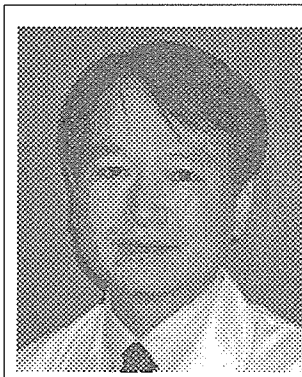
Hiroyuki Takano · Yingjie Qin · Hiroshi Hasegawa · Kazutaka Ueda · Yuriko Niitsuma · Masashi Ohtsuka · Issei Komuro

Effects of G-CSF on left ventricular remodeling and heart failure after acute myocardial infarction

Received: 26 September 2005 / Accepted: 7 November 2005 / Published online: 17 January 2006

Abstract Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic cytokine that promotes proliferation and differentiation of neutrophil progenitors. G-CSF also possesses immunomodulatory properties. G-CSF-induced hematopoietic stem cell mobilization is widely used clinically for transplantation. After it was recently reported that G-CSF mobilizes bone marrow stem cells (BMSCs) into the infarcted hearts and accelerates the differentiation into vascular cells and cardiac myocytes, myocardial regeneration utilizing mobilization of BMSCs by G-CSF is attracting the attention of investigators. In animal models, G-CSF prevents left ventricular remodeling and dysfunction after acute myocardial infarction, at least in part, through a decrease in apoptotic cells and an increase in vascular cells. Although it is controversial whether BMSCs mobilized by G-CSF can differentiate into cardiac myocytes, G-CSF-induced angiogenesis is indeed recognized in infarcted heart. The cardioprotective effects of G-CSF are recognized even in isolated perfused heart. In addition, G-CSF activates various signaling pathways such as Akt, extracellular signal-regulated kinase, and Janus kinase 2/signal transducer and activator of transcription 3 through G-CSF receptors in cardiac myocytes. These observations 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 injured myocardium. Although the effects of G-CSF on the progression of atherosclerosis are still unclear, there is a possibility that G-CSF will become a promising therapy for ischemic heart diseases.

H. Takano · Y. Qin · H. Hasegawa · K. Ueda · Y. Niitsuma · M. Ohtsuka · I. Komuro (✉)
Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba, 260-8670, Japan
e-mail: komuro-tky@umin.ac.jp
Tel.: +81-43-2262097
Fax: +81-43-2262557



HIROYUKI TAKANO received his M.D. and Ph.D. degrees from Chiba University School of Medicine, Japan. He is presently a scientist and physician in Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, Chiba, Japan. His current research interests include elucidation of mechanism underlying heart failure and development of novel therapy.

ISSEI KOMURO received his M.D. and Ph.D. degrees from University of Tokyo School of Medicine, Japan. He is currently Professor in Medicine, Chairman of Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, Chiba, Japan. His research interests are mechanisms of heart failure and cardiovascular regeneration.

Keywords Angiogenesis · Cytokine · G-CSF · Heart failure · Myocardial infarction · Remodeling

Abbreviations AMI: Acute myocardial infarction · BMSC: Bone marrow stem cell · EC: Endothelial cell · EPC: Endothelial progenitor cell · ERK: Extracellular signal-regulated kinase · G-CSF: Granulocyte colony-stimulating factor · G-CSFR: G-CSF receptor · GFP: Green fluorescent protein · HSC: Hematopoietic stem cell · Jak: Janus kinase · LV: Left ventricle · PCI: Percutaneous coronary intervention · SCF: Stem cell factor · SDF-1: Stromal cell-derived factor-1 · STAT: Signal transducer and activator of transcription · VEGF: Vascular endothelial growth factor · VSMC: Vascular smooth muscle cell

Introduction

Therapeutic advances have improved survival of patients with acute myocardial infarction (AMI). As the clinical outcome after AMI depends on the extent of damaged myocardium, myocardial salvage induced by reperfusion therapy such as thrombolysis or percutaneous transluminal coronary intervention (PCI) is very important in treatment of patients with AMI. Although the progress of PCI device has reduced mortality, these therapies have led to a dramatic increase in the number of patients suffering from heart failure [1, 2]. Recently, medical interest in regeneration has grown and researches on gene and cell-based therapies have been concentrated. Several hematopoietic cytokines including interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor, stem cell factor (SCF), and erythropoietin have been known to regulate the growth and differentiation of hematopoietic progenitor cells. These cytokines have also effects to mobilize bone marrow stem cells (BMSCs) [3]. It has been recently demonstrated that BMSCs differentiate into cardiac myocytes, endothelial cells (ECs), and vascular smooth muscle cells (VSMCs) in mouse model of AMI [4]. In addition, cytokine-mediated recruitment of BMSCs has been reported to improve cardiac dysfunction and reduce mortality after AMI in mice [5]. Afterward, we and other groups reported that G-CSF prevents left ventricular (LV) remodeling and dysfunction after AMI in various animal models [6–11]. It seems that the beneficial effects of G-CSF are attributed to direct action on injured myocardium rather than to differentiation of BMSCs into cardiac myocytes. In this study, we review the biological functions of G-CSF and discuss the therapeutic potential of G-CSF for AMI.

Myocardial infarction and cardiac remodeling

Therapeutic advances have improved the survival of patients with AMI. As clinical outcome after AMI depends on the extent of damaged myocardium, myocardial salvage induced by reperfusion therapies such as thrombolytic agents and PCI confers a benefit on the patients. However, the improved survival of patients with MI has led to an increase in the number of patients suffering from heart failure [1, 2]. Congestive heart failure, which is mostly caused by MI, remains one of the major causes of mortality in the Western world. Despite the development of pharmacological and mechanical revascularization techniques, heart failure proceeds as a consequence of MI. Therefore, more effective treatment options for patients with AMI need to be developed.

The term LV remodeling was previously described as a physiologic and pathologic condition that occurs after AMI. LV remodeling consists of the process including cardiomyocyte loss due to necrosis or apoptosis, cardiomyocyte lengthening, LV wall thinning, infarct expansion, LV dilation, cardiomyocyte hypertrophy, and collagen accumulation [2]. Complex architectural alterations are induced in both infarcted and noninfarcted myocardium after AMI. Dilatation of LV and infarcted wall thinning are the prominent features in the infarcted region. In addition, LV remodeling with compensatory dilation and hypertrophy is induced in the noninfarcted region. Cardiac remodeling is also regarded as a process occurring in other cardiovascular diseases such as hypertension, valvular heart disease, myocarditis, and dilated cardiomyopathy [12]. The process of cardiac remodeling is influenced by hemodynamic load, neurohumoral activation, and other factors [12]. Although cardiac remodeling is initially an adaptive response to maintain normal function, it gradually becomes maladaptive and subsequently leads to progressive decompensation and congestive heart failure. The degree of post-MI remodeling roughly depends on the infarcted size. Large infarction induces greater dilation of LV and more increase in wall stress of LV than small infarction. Although cardiac myocytes play critical roles in the remodeling process, cardiac fibroblasts, coronary vasculature, and extracellular matrix (ECM) are also involved in the process [1, 2, 12].

Many mediators such as neurohumoral factors, cytokines, growth factors, and enzymes are known to be involved in the progression of cardiac remodeling process. Accumulating evidence has suggested that neurohumoral factors such as angiotensin II (Ang II), aldosterone, endothelin-1 (ET-1), and norepinephrine play pivotal roles in the development of LV remodeling [1, 2, 12]. In particular, activation of the local renin–angiotensin system (RAS) in myocardium is important in the process of cardiac remodeling. Many components of RAS including angiotensinogen, angiotensin-converting enzyme (ACE), and Ang II type 1 receptor are upregulated in the heart after MI. Ang II induces hypertrophy of cardiac myocytes and increases proliferation of cardiac fibroblasts and collagen synthesis. ET-1 is also involved in the remodeling process including cardiac hypertrophy and collagen synthesis. Cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 also play important roles in cardiac remodeling. Cytokines are secreted in response to a variety of stimuli. Growth factors such as transforming growth factor (TGF)-β, fibroblast growth factor, and platelet-derived growth factor are also associated with remodeling. TGF-β1 plays an important role in the regulation of ECM production and fibrosis. Enzymes such as matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) have been recently shown to be involved in the remodeling process [1, 2, 12]. The degradation of ECM within the myocardium plays an important role in the progression of cardiac remodeling and heart failure after MI [13]. MMPs are a family of proteolytic enzymes for ECM degradation and are involved in tissue remodeling processes including morphogenesis and wound healing (Fig. 1).

Cardiomyocyte death, which is induced by necrosis or apoptosis, is an important cause of cardiac remodeling. Apoptosis, known as programmed cell death, is a fundamental physiologic and pathologic mechanism of cell death

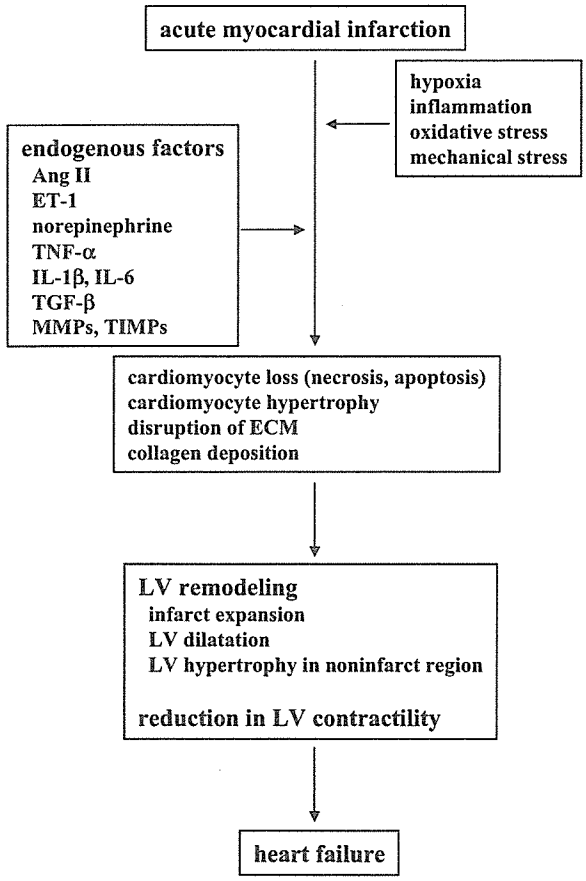


Fig. 1 Cardiac remodeling. *Ang II* Angiotensin II, *ECM* extracellular matrix, *ET-1* endothelin-1, *IL* interleukin, *LV* left ventricle, *MMPs* matrix metalloproteinases, *TGF-β* transforming growth factor-β, *TIMPs* tissue inhibitors of metalloproteinases, *TNF-α* tumor necrosis factor-α

during life. Myocardial apoptosis peaks at 4–12 h after AMI and is persistently detected up to 10 days in rats [14]. In acute phase of MI, apoptosis has been reported to represent the major form of cardiomyocyte death. Persistent apoptosis still occurs in the infarcted region even during the subacute phase (up to 60 days) in human MI heart [14]. Moreover, a strong correlation between apoptotic rate and degree of LV remodeling was recognized in the phase.

Therefore, preventing the remodeling process is an important therapeutic approach for heart failure after AMI. Therapeutic agents such as ACE inhibitors and β-blockers are used to reduce cardiac remodeling and to decrease morbidity and mortality in patients with heart failure. Although cardiac myocytes are believed to be terminally differentiated cells and have inability to regenerate, it was recently demonstrated that cardiac myocytes proliferate after MI in humans [15]. Therefore, there is a possibility that enhancement of regeneration of cardiac myocytes as well as stimulation of neovascularization may prevent cardiac remodeling and progression of heart failure. As the reduction in number of cardiomyocytes under pathologic conditions causes LV dilatation and hypertrophy of the remaining cardiomyocytes, myocardial regeneration seems to become a promising strategy for cardiac remodeling.

Myocardial regeneration by stem-cell-based therapy

BMSCs have been recently expected as a potential tool in regenerative medicine. Orlic et al. [4] have demonstrated that BMSCs differentiate into cardiac myocytes, ECs, and VSMCs in adult mouse model of AMI. They injected male BMSCs (Lin^- c-kit^+) that carried the gene encoding enhanced green fluorescent protein (eGFP) into the myocardium near the site of infarction within 5 h after ligation of left coronary artery in adult female mouse. After 9 days, regenerating myocardium was observed. These regions consisted of Y-positive $eGFP^+$ cardiac myocytes and small coronary vessels. Regeneration of myocardium was not recognized in hearts that were injected with Lin^- c-kit^- bone marrow cells that are conceived to be devoid of stem cells, suggesting that hematopoietic stem cells (HSCs), as opposed to other BM resident cells, were responsible for the regeneration of myocardium. Newly formed myocardium surprisingly occupied 68% of the infarcted region of ventricle. Furthermore, they examined whether cytokine increases BMSCs mobilization to injured myocardium and promotes myocardial regeneration [5]. After mice were injected with rat SCF and recombinant human G-CSF once a day for 5 days, mice were supposed to ligation of left coronary artery. SCF and G-CSF were given for 3 more days. Cytokine-mediated mobilization of BMSCs resulted in myocardial regeneration characterized by dividing cardiac myocytes and forming vascular structures 27 days after AMI [5]. This method significantly reduced mortality and improved cardiac function such as ejection fraction, LV diameter, and LV pressure. Although their results suggest that locally delivered BMSCs could generate new myocardium and then improve prognosis of the patients with AMI, recent studies have demonstrated that adult HSCs do not transdifferentiate into cardiac myocytes in murine AMI model [16, 17]. Kamihata et al. [18] injected bone marrow-derived mononuclear cells labeled with GFP into the ischemic border and infarcted zone immediately after MI in pig. GFP signal was detected in capillaries, but not in cardiac myocytes, and myocardial blood flow was improved 3 weeks later. The blood concentration of $CD34^+$ mononuclear cells, presumably endothelial progenitor cells (EPCs) and their putative precursors, has been reported to be significantly increased in patients with MI, peaking on day 7 after onset [19]. These findings suggest that BMSCs can differentiate into vascular cells but not cardiac myocytes in MI heart. Kajstura et al. [20] have provided quite recently the evidence supporting their initial observations that BMSCs, when properly administrated in the infarcted heart, differentiate into cardiomyocytes. They raise several possibilities accounting for these contrasting results. The most likely possibility is a technical difference in the experimental protocol. The controversy on this issue is described in detail in a recent review [21].

G-CSF-induced signaling pathways and hematopoietic effects

G-CSF binds to a cell surface receptor that is a member of cytokine receptor superfamily [22]. G-CSF receptor (G-CSFR) is expressed on myeloid progenitor cells, myeloid leukemia cells, leukemic cell lines, mature neutrophils, platelets, monocytes, and some lymphoid cell lines [22]. In addition, G-CSFR has been detected on several nonhematopoietic cell types, including ECs, placenta, trophoblastic cells, and some small cell lung carcinoma cell lines. Calhoun et al. [23] have reported that both G-CSF and G-CSFR are present in nearly every organ in human fetal tissues. However, it has not been elucidated whether G-CSFR is expressed on cardiac myocytes in the adult heart.

G-CSF activates a variety of intracellular signaling cascades such as Janus kinase (Jak)-signal transducer and activator of transcription (STAT), Ras-Raf-mitogen-activated protein (MAP) kinase, and Src family kinase pathways [22]. In proliferating cells, ligand binding induces homodimerization of G-CSFR and activation of associated Jak tyrosine kinases. Activation of Jak results in tyrosine phosphorylation of G-CSFR and activation of STAT transcription factors. Activated STATs translocate to the nucleus and induce gene transcription. Activation of G-CSFR also induces tyrosine phosphorylation of SH2-containing protein Shc. Binding and tyrosine phosphorylation of Shc is associated with activation of the Ras pathway and MAP kinases, and induction of immediate-early genes. In nonproliferating terminally differentiated cells, G-CSF activates different signaling pathways that do not appear to involve any of the known Jak-STAT pathway or Ras-MAP kinase pathway. Another signaling pathway that is activated by G-CSF is phosphatidylinositol (PI) 3-kinase [24]. Serine/threonine kinase Akt was identified as a downstream target of PI3-kinase. Activation of Akt has been shown to be a critical component of cell survival signaling.

G-CSF has been also reported to exert an anti-inflammatory effect [25]. G-CSF attenuates release of proinflammatory cytokines such as TNF-α, IL-1β, and IL-12 in lipopolysaccharide (LPS)-stimulated whole blood [26]. This modulation of LPS-induced cytokine release occurs when monocytes are incubated with G-CSF in vitro or ex vivo in the blood of G-CSF-treated volunteers. In a cross-over design study, volunteers were administered with G-CSF (5 μg/kg) subcutaneously 24 h before a low dose of endotoxin challenge [27]. G-CSF attenuated serum levels of TNF-α and IL-6 compared with placebo treatment. G-CSF increased survival rate after experimental abdominal contamination and infection model in rat through reduction of TNF-α and IL-6 levels [28].

Development of hematopoietic cells throughout adult life occurs within bone marrow microenvironment. Whereas mature cells are continuously released into blood circulation, a small pool of undifferentiated stem cells and progenitor cells are maintained within bone marrow [29]. G-CSF causes a marked increase in the release of HSCs into the periphery [30, 31]. Kocher et al. examined whether injection of G-CSF-mobilized adult human $CD34^+$ cells in the tail vein induces angiogenesis of infarcted myocardium in rats [32]. Labeled human $CD34^+$ cells were present in the infarcted zone but not in unaffected myocardium or sham-operated myocardium, implying that an injury was required for the localization. G-CSF-mobilized $CD34^+$ cells induced neoangiogenesis in the infarcted region, thus preventing apoptosis of cardiac myocytes and reducing collagen deposition and scar formation after MI.

Wright et al. [33] demonstrated that purified adult murine HSCs migrate to stromal cell-derived factor-1 (SDF-1) and not to any other known chemokines revealing a central role for SDF-1/CXCR4 interactions in adult murine hematopoiesis. SDF-1 seems to be a key regulator of murine HSCs migration, homing, and anchorage of repopulating cells to bone marrow as well as release of maturing cells into the blood circulation. Although several reports have shown that a newly formed gradient of SDF-1 towards blood circulation is sufficient to induce stem cell mobilization, the mechanism by which G-CSF induces HSC mobilization was not fully understood. Petit et al. have demonstrated that G-CSF induced a reduction of SDF-1 and an increase in its receptor CXCR4 in bone marrow, whereas their protein expression in blood was less affected [34]. The decrease in bone marrow SDF-1 due to its degradation by neutrophil elastase correlated with stem cell mobilization. Mobilization of HSCs was inhibited by elastase inhibition or by neutralizing CXCR4 or SDF-1 antibodies. These data suggest that SDF-1/CXCR4 signaling plays an important role in stem cell mobilization. It is interesting to note that expression of SDF-1 in infarcted myocardium is upregulated immediately after AMI and then downregulated within 7 days [35].

Cardioprotective effects of G-CSF on acute myocardial infarction

As mentioned above, Orlic et al. [5] has reported that the combination therapy with G-CSF and SCF significantly improves cardiac function and reduces mortality after AMI in mice. However, the cytokine treatment was started before MI and clinically relevant protocol involving post-MI administration of G-CSF alone was not examined. Therefore, we examined whether the single treatment with G-CSF that started after AMI has also beneficial effects [6]. MI mice were produced by ligation of left coronary artery and divided into the following four groups: (1) administration of vehicle (control group), (2) administration of G-CSF (100 μg/kg/day) and SCF (200 μg/kg/day) from 5 days before MI through 3 days after (pre-GS group), (3) administration of G-CSF (100 μg/kg/day) and SCF (200 μg/kg/day) for 5 days after MI (post-GS group), and (4) administration of G-CSF (100 μg/kg/day) alone for 5 days after MI (post-G group). In post-GS and post-G groups, first injection of vehicle, G-CSF, or SCF was subcutaneously given at 2 h after MI. All the three

treatment groups with G-CSF showed less LV remodeling and improved cardiac function and survival rate after MI [6]. The number of apoptotic cells was decreased in the border area of all the treatment groups with G-CSF. We next used mice that were replaced by BM cells of eGFP-expressing mice to elucidate the role of BM cells in the improvement of cardiac function. Many GFP-positive cells were recognized in the border area of all the three treatment groups but not the control group. Most of the GFP-positive cells were infiltrated blood cells and some GFP-positive cells were observed at capillary walls [6]. There were few GFP-positive cardiomyocytes in the border area as well as in the infarcted area and the remote area. Therefore, we next compared the number of vessels in the border area. The number of capillaries in the border area after MI was much greater in all the three treatment groups than in the control group. Even if the cytokine treatment is started after MI, it could prevent LV remodeling and dysfunction after MI at least in part through an increase in neovascularization and a decrease in apoptosis in the border area.

We subsequently examined as a preclinical study whether G-CSF treatment is effective in preventing cardiac remodeling after MI in large animals [9]. MI was produced by ligation of left anterior descending coronary artery in swine. G-CSF (10 μg/kg/day) was injected subcutaneously from 24 h after the ligation for 7 days. Echocardiographic examination revealed that G-CSF treatment improved cardiac dysfunction and reduced LV remodeling at 4 weeks after MI [9]. In the ischemic region, the number of apoptotic ECs was smaller and the number of vessels was larger in the G-CSF treatment group than in the control group. Moreover, vascular endothelial growth factor (VEGF) was more abundantly expressed and Akt was more strongly activated in the ischemic region of the G-CSF treatment group than of the control group. As Akt has been reported to play an important role in cell survival and angiogenesis, Akt seems to be important in the cardioprotective effects of G-CSF. These findings suggest that G-CSF prevents cardiac dysfunction and remodeling after MI in large animals. It is interesting to note that there was a difference in LV function between the G-CSF treatment group and the control group already at 1 week after MI. The finding was not explained simply by G-CSF-induced mobilization of BMSCs into the injured myocardium. So we speculated that G-CSF could not only mobilize BMSCs but also act directly on myocardium in MI hearts. As mentioned above, it has been reported that G-CSFR is expressed on various blood cells but whether G-CSFR is expressed on cardiomyocytes has yet been determined. We detected the expression of G-CSFR messenger ribonucleic acid (mRNA) in both adult mouse heart and cultured neonatal mouse cardiomyocyte by reverse transcription-polymerase chain reaction [10]. Furthermore, we recognized the expression of G-CSFR protein in neonatal rat cardiomyocyte by immunocytochemical analysis. We next examined whether G-CSF induces the activation of intracellular signaling molecules in cardiomyocytes. G-CSF (100 ng/ml) significantly activated Jak2, STAT1, and STAT3 in a dose-dependent manner [10]. These results suggest that G-CSFR is expressed on cardiomyocyte and G-CSF induces the activation of signaling molecules through G-CSFR.

We further examined whether G-CSF has direct effect on the myocardium after ischemia-reperfusion injury by using Langendorff-perfused heart model. The isolated hearts underwent 30-min ischemia followed by 120-min reperfusion with perfusate containing G-CSF (300 ng/ml) or vehicle. G-CSF significantly reduced the infarct size measured by triphenyltetrazolium chloride staining [10]. LV developed pressure in G-CSF group was significantly better than that in control group at 120 min after reperfusion [10]. Western blot analysis has demonstrated that G-CSF significantly increased the phosphorylation of Akt, Jak2, STAT3, and extracellular signal-regulated kinase in the hearts subjected to ischemia followed by 7 min of reperfusion, and that the infarct reduction afforded by G-CSF administration is abolished in the presence of Akt inhibitor LY294002 or Jak2 inhibitor AG490 but not MEK inhibitor PD98059 (unpublished data). As there are neither blood cells nor inflammatory mediators in this experimental model, G-CSF is regarded to have direct effects on myocardium.

Other groups also examined the effects of G-CSF on MI hearts in animal models. Minatoguchi et al. [7] demonstrated that G-CSF prevented cardiac remodeling and dysfunction at 3 months in ischemia-reperfusion rabbit model. G-CSF increased the number of macrophages in the infarcted area at 2 days after MI and the expression levels of MMP-1 and MMP-9 in the ischemic region at 7 days after MI. They suggested that G-CSF had beneficial effects on MI hearts through acceleration of healing process and myocardial regeneration. Sugano et al. [11] reported that G-CSF attenuated early ventricular expansion after AMI in rats. Expression levels of TGF-β and procollagen type I and type III mRNA in the infarcted area at 3 days were higher in G-CSF group than control group. Accumulation of collagen in the infarcted area at 7 days was more prominent in G-CSF group than control group. Kawada et al. [8] demonstrated that nonhematopoietic mesenchymal stem cells (MSCs) in bone marrow are mobilized into the infarcted area and differentiated into cardiomyocytes by G-CSF after MI in mice. In contrast to those reports, some studies did not recognized the beneficial effects of G-CSF in AMI model [36, 37]. This discrepancy is not yet understood.

Ischemic cardiomyopathy is a leading cause of congestive heart failure in many countries [38]. In hibernating myocardium, contractile function is depressed because of reduced myocardial perfusion [39]. The viable but dysfunctional myocardium could be reversed by restoration of myocardial blood flow [40]. Although interventional therapies such as coronary artery bypass grafting and PCI are performed to increase blood supply to ischemic region, many patients with ischemic cardiomyopathy cannot be treated due to severe and diffuse coronary atherosclerosis. Therefore, we further examined whether G-CSF treatment is effective on chronic hibernating myocardium in swine [41]. G-CSF improved cardiac function of chronic myocardial ischemia through decreases in fibrosis and apo-

ptotic death, and an increase in vascular density in the ischemic region [41]. Akt was more strongly activated in the hearts of the G-CSF group than the control group. There is a possibility that G-CSF could prevent the progression of LV remodeling in hibernating myocardium as well as infarcted myocardium.

STAT3 as a key molecule in G-CSF-induced cardioprotective effects

As it is well known that apoptotic cell death is increased immediately after AMI, we examined the effect of G-CSF on hydrogen peroxide (H_2O_2)-induced apoptosis of cardiomyocytes. Rat neonatal cardiomyocytes ware exposed to H_2O_2, and apoptotic cell death by staining annexin V was detected. Pretreatment with G-CSF significantly decreased the number of H_2O_2-induced apoptosis of cardiomyocytes [10]. Although expression levels of anti-apoptotic proteins such as Bcl-2 and Bcl-xL, which are target molecules of Jak-STAT pathway, were decreased after stimulation with H_2O_2, the change was inhibited by pretreatment with G-CSF. AG490, an inhibitor of Jak2, abolished the upregulation of Bcl-2 expression level by G-CSF. Adenovirus transfection of dominant-negative STAT3 (dnSTAT3) to cardiomyocytes inhibited the protective effect of G-CSF [10]. These results suggest that G-CSF may inhibit apoptosis of cardiomyocytes through Jak2-STAT3 pathway. STAT3 is a member of STAT family of transcription factors and activated by numerous growth factors and cytokines including IL-6, leukemia inhibitory factor (LIF), and cardiotrophin-1 through the shared receptor glycoprotein 130 (gp130). Upon receptor activation, STAT3 is phosphorylated and activated by Jak1 or Jak2. Several studies demonstrated that STAT3 protects the heart against pathophysiologic stress such as ischemia, mechanical stress, and cytotoxic agents. Therefore, we next analyzed using transgenic mice with cardiac-specific overexpression of dnSTAT3 (dnSTAT3-TG) whether STAT3 is involved in the cardioprotective effects of G-CSF in vivo. Although there were no significant differences in LV function and size between wild-type mice (WT) and dnSTAT3-TG at basal level, the cardioprotective effects of G-CSF on post-MI hearts were abolished in dnSTAT3-TG [10]. Expression levels of Bcl-2 and Bcl-xL in MI hearts of WT but not dnSTAT3-TG were increased by G-CSF treatment. Although G-CSF similarly increased the number of stem cells (double-positive cells for c-kit and Sca-1) in peripheral blood in WT and dnSTAT3-TG, G-CSF did not increase cardiac homing of bone marrow cells in both groups. G-CSF did not affect the number of cardiac stem cells, which exist in Sca-1-positive populations of adult myocardium, in MI hearts of both WT, and dnSTAT3-TG. These results suggest that the beneficial effects of G-CSF on MI hearts may be attributed to direct action on myocardium rather than mobilization and differentiation of stem cells.

Various studies have shown the involvement of STAT3 in cardiovascular diseases. It has been demonstrated that the expression level of STAT3 in myocardium was significantly decreased in patients with end-stage dilated cardiomyopathy (DCM) [42]. Transgenic mice with cardiac-specific overexpression of constitutively active STAT3 (caSTAT3-TG) presented increased capillary density accompanied by enhanced expression of VEGF in hearts [43]. Infarct size in hearts of caSTAT3-TG was significantly reduced compared with WT after ischemia–reperfusion injury [44]. Reactive oxygen species (ROS) generated in the reperfused myocardium lead to cell damage and heart failure. The level of ROS production was less in hearts of caSTAT3-TG than WT, and ROS scavengers, metallothionein1 and metallothionenin2, were upregulated in hearts of caSTAT3-TG [44]. These results raise the possibility that G-CSF may increase coronary blood flow and ameliorate heart failure through upregulation of STAT3 even in non-ischemic cardiomyopathy such as DCM. It is well known that long-term therapy with doxorubicin, an antitumor drug, induces irreversible cardiomyopathy [45]. Doxorubicin-induced cardiomyopathy is histopathologically associated with loss of myofibrils, distension of sarcoplasmic reticulum, and vacuolization of cytoplasm. Although redox, ROS, and mitochondrial dysfunction are proposed as the pathogenesis of doxorubicin-induced cardiac toxicity, the precise mechanism has not been fully elucidated. It was previously reported that the treatment with doxorubicin reduced STAT3 mRNA level of myocardium and led to an increase in mortality due to congestive heart failure in mice [46]. Overexpression of STAT3 in cardiomyocytes provided not only cardiac hypertrophy but also protection from doxorubicin-induced cardiac toxicity [46]. These results suggest that G-CSF may protect the heart from doxorubicin through upregulation of STAT3 in cardiomyocytes. We have recently reported that intramuscular injection of LIF plasmid DNA induces regeneration of myocardium and prevents cardiac dysfunction after AMI in mice [47]. As LIF is also known to activate STAT3 through gp130, our results reinforce the hypothesis that STAT3 may be important in cardioprotection from pathophysiologic stress.

Clinical trial

Based on the experimental data in animal models, clinical trials evaluating the feasibility and safety of G-CSF in patients with AMI were recently carried out. Kang et al. [48] found an unexpectedly high rate of in-stent restenosis in patients with AMI or old MI who were subjected to subcutaneous injections of G-CSF (10 μg/kg) for 4 days before PCI. In their study, the patients did not receive primary PCI during the golden time of AMI treatment and were treated with G-CSF for 4 days before PCI. Only a few patients, three patients in G-CSF group and one patient in control group, were assessed by coronary angiography at 6 months follow-up. To elucidate the safety of G-CSF therapy on patients with atherosclerosis, we studied the effect of G-CSF using two kinds of rabbit models of atherosclerosis, Watanabe heritable hyperlipidemic (WHHL) rabbits and vascular injury model. WHHL rabbits

were randomly treated with G-CSF (100 μg/kg/day s.c. for 7 days) or saline, beginning at 14 months old and continuing daily for 7 days. At 4 weeks after the treatment, the coronary arteries and aorta were evaluated. The treatment with G-CSF significantly reduced the stenosis score of coronary artery in WHHL rabbits, and the lipid plaque area of thoracic aorta was decreased by the treatment with G-CSF group (unpublished data). The vascular injury model was created by inflating angioplasty balloon in the iliac artery of Japanese white rabbits and was divided into G-CSF group (100 μg/kg/day s.c. for 7 days) and control group. In the vascular injury model, the treatment with G-CSF significantly prevented the increase in neointima/media ratio at 4 weeks after the injury and accelerated the reendothelialization of denuded vessels at 1 week (unpublished data). Kong et al. [49] also demonstrated that G-CSF treatment accelerated reendothelialization and inhibited neointimal thickening in balloon-injured arteries through an increase in the number of EPCs in rat model. These observations suggest that G-CSF can be used safely in patients with atherosclerosis.

Afterward, contrary consequences by other groups were recently reported. Valgimigli et al. [50] examined the effects and safety of G-CSF (5 μg/kg for 4 days) in patients with AMI. Twenty patients with AMI, of whom 14 underwent PCI with stent implantation, were randomized to G-CSF group or placebo group. They showed that the relative increases in LV ejection fraction and LV end-diastolic volume tend to be higher and lower, respectively, in G-CSF group compared with control group at 6 months follow-up. One patient in control group and no patients in G-CSF group presented binary restenosis at 6-month follow-up coronary angiography. Kuethe et al. [51] examined 23 patients with AMI in a nonrandomized trial. They demonstrated that treatment with G-CSF (10 μg/kg for 7 days) significantly improved regional wall motion, myocardial perfusion, and ejection fraction at 3 months follow-up. No severe side effects of G-CSF were observed. Jorgensen et al. [52] performed angiography and intravascular ultrasound (IVUS) at 5 months follow-up in 41 patients in the ST-elevation myocardial infarction (STEMI) trial, which is a randomized placebo-controlled double-blind trial on the effects of G-CSF (10 μg/kg for 6 days) in the functional recovery of infarcted myocardium. There were no differences in angiographic in-segment restenosis (>50% diameter stenosis) and in-stent neointimal hyperplasia determined by IVUS between G-CSF group (n=20) and placebo group (n=21). They concluded that G-CSF treatment does not increase the risk of in-stent restenosis in AMI patients treated with PCI.

Conclusion and future prospects

Many experimental data suggest that treatment with G-CSF might become a novel therapeutic strategy for AMI. It is conceivable that anti-apoptotic and angiogenic effects of G-CSF are effective for LV remodeling after AMI (Fig. 2). When we perform clinical trial to assess feasibility and safety of novel therapies including cell, gene, and cytokine therapies for coronary heart diseases, we must strictly determine the inclusion criteria of patients. Patients with severe coronary lesions or unstable vital signs are not eligible for those trials. Although recent clinical trials suggest feasibility and safety of G-CSF treatment in AMI, safety in the long term has not yet been determined. It has

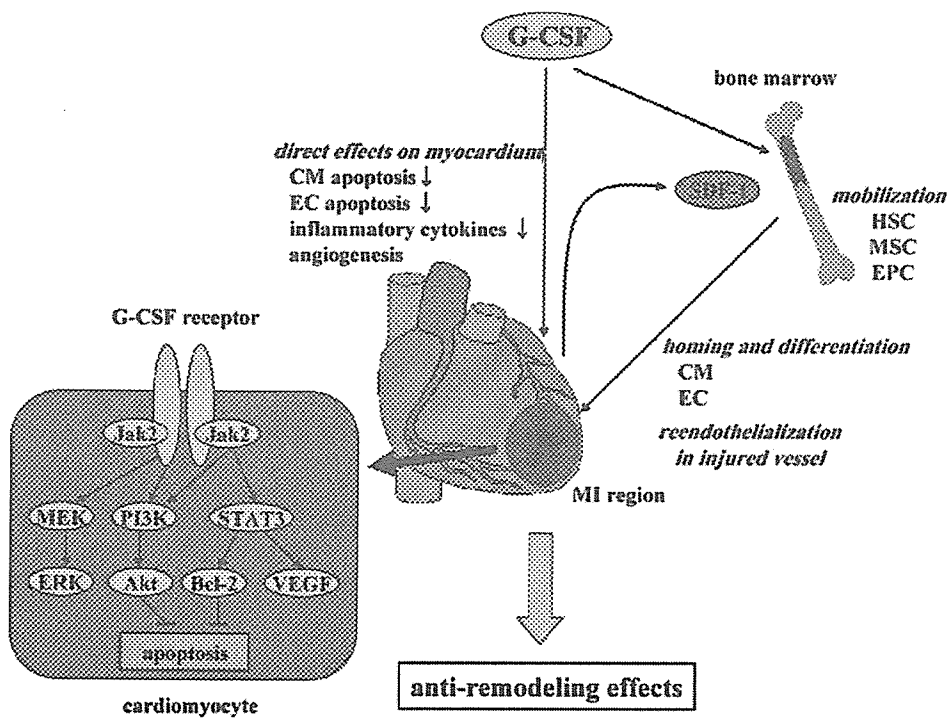


Fig. 2 Hypothetical scheme demonstrating the mechanisms of cardioprotection induced by G-CSF. *CM* Cardiomyocyte, *EC* endothelial cell, *EPC* endothelial progenitor cell, *ERK* extracellular signal-regulated kinase, *HSC* hematopoietic stem cell, *Jak2* Janus kinase 2, *MEK* ERK kinase, *MI* myocardial infarction, *MSC* mesenchymal stem cell, *PI3K* phosphatidylinositol 3-kinase, *SDF-1* stromal cell-derived factor-1, *STAT3* signal transducer and activator of transcription 3, *VEGF* vascular endothelial growth factor

not been identified how much dose of G-CSF should be used and when the treatment should be started. Further studies are needed to establish the most effective and the less adverse regimen of G-CSF treatment.

References

1. Jessup M, Brozena S (2003) Heart failure. N Engl J Med 348:2007–2018
2. Nian M, Lee P, Khaper N, Liu P (2004) Inflammatory cytokines and postmyocardial infarction remodeling. Circ Res 94:1543–1553
3. Papayannopoulou T (2004) Current mechanistic scenarios in hematopoietic stem/progenitor cell mobilization. Blood 103: 1580–1585
4. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P (2001) Bone marrow cells regenerate infarcted myocardium. Nature 410:701–705
5. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P (2001) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 98:10344–10349
6. Ohtsuka M, Takano H, Zou Y, Toko H, Akazawa H, Qin Y, Suzuki M, Hasegawa H, Nakaya H, Komuro I (2004) Cytokine therapy prevents left ventricular remodeling and dysfunction after myocardial infarction through neovascularization. FASEB J 18:851–853
7. Minatoguchi S, Takemura G, Chen XH, Wang N, Uno Y, Koda M, Arai M, Misao Y, Lu C, Suzuki K, Goto K, Komada A, Takahashi T, Kosai K, Fujiwara T, Fujiwara H (2004) Acceleration of the healing process and myocardial regeneration may be important as a mechanism of improvement of cardiac function and remodeling by postinfarction granulocyte colony-stimulating factor treatment. Circulation 109:2572–2580
8. Kawada H, Fujita J, Kinjo K, Matsuzaki Y, Tsuma M, Miyatake H, Muguruma Y, Tsuboi K, Itabashi Y, Ikeda Y, Ogawa S, Okano H, Hotta T, Ando K, Fukuda K (2004) Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood 104:3581–3587
9. Iwanaga K, Takano H, Ohtsuka M, Hasegawa H, Zou Y, Qin Y, Odaka K, Hiroshima K, Tadokoro H, Komuro I (2004) Effects of G-CSF on cardiac remodeling after acute myocardial infarction in swine. Biochem Biophys Res Commun 325:1353–1359
10. Harada M, Qin Y, Takano H, Minamino T, Zou Y, Toko H, Ohtsuka M, Matsuura K, Sano M, Nishi J, Iwanaga K, Akazawa H, Kunieda T, Zhu W, Hasegawa H, Kunisada K, Nagai T, Nakaya H, Yamauchi-Takihara K, Komuro I (2005) G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. Nat Med 11:305–311
11. Sugano Y, Anzai T, Yoshikawa T, Maekawa Y, Kohno T, Mahara K, Naito K, Ogawa S (2005) Granulocyte colony-stimulating factor attenuates early ventricular expansion after experimental myocardial infarction. Cardiovasc Res 65:446–456
12. Takano H, Hasegawa H, Nagai T, Komuro I (2003) Implication of cardiac remodeling in heart failure: mechanisms and therapeutic strategies. Intern Med 42:465–469
13. Jugdutt BI (2003) Ventricular remodeling after infarction and the extracellular collagen matrix: when is enough enough? Circulation 108:1395–1403
14. Abbate A, Biondi-Zoccai GG, Baldi A (2002) Pathophysiologic role of myocardial apoptosis in post-infarction left ventricular remodeling. J Cell Physiol 193:145–153
15. Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P (2001) Evidence that human cardiac myocytes divide after myocardial infarction. N Engl J Med 344:1750–1757
16. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ (2004) Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 428:664–668
17. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC (2004) Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 428: 668–673
18. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A, Shintani S, Murohara T, Imaizumi T, Iwasaka T (2001) Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. Circulation 104:1046–1052
19. Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T (2001) Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation 103:2776–2779
20. Kajstura J, Rota M, Whang B, Cascapera S, Hosoda T, Bearzi C, Nurzynska D, Kasahara H, Zias E, Bonafe M, Nadal-Ginard B, Torella D, Nascimbene A, Quaini F, Urbanek K, Leri A, Anversa P (2005) Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. Circ Res 96:127–137
21. Leri A, Kajstura J, Anversa P (2005) Cardiac stem cells and mechanisms of myocardial regeneration. Physiol Rev 85:1373–1416
22. Avalos BR (1996) Molecular analysis of the granulocyte colony-stimulating factor receptor. Blood 88:761–777
23. Calhoun DA, Donnelly WH Jr, Du Y, Dame JB, Li Y, Christensen RD (1999) Distribution of granulocyte colony-stimulating factor (G-CSF) and G-CSF-receptor mRNA and protein in the human fetus. Pediatr Res 46:333–338
24. Dong F, Larner AC (2000) Activation of Akt kinase by granulocyte colony-stimulating factor (G-CSF): evidence for the role of a tyrosine kinase activity distinct from the Janus kinases. Blood 95:1656–1662
25. Hartung T (1998) Anti-inflammatory effects of granulocyte colony-stimulating factor. Curr Opin Hematol 5:221–225
26. Boneberg EM, Hareng L, Gantner F, Wendel A, Hartung T (2000) Human monocytes express functional receptors for granulocyte colony-stimulating factor that mediate suppression of monokines and interferon-gamma. Blood 95:270–276
27. Pajkrt D, Manten A, van der Poll T, Tiel-van Buul MM, Jansen J, Wouter ten Cate J, van Deventer SJ (1997) Modulation of cytokine release and neutrophil function by granulocyte colony-stimulating factor during endotoxemia in humans. Blood 90: 1415–1424
28. Bauhofer A, Stinner B, Kohlert F, Reckzeh B, Lorenz W, Celik I (2002) Granulocyte colony-stimulating factor but not peritoneal lavage increases survival rate after experimental abdominal contamination and infection. Br J Surg 89:1457–1464
29. Morrison SJ, Uchida N, Weissman IL (1995) The biology of hematopoietic stem cells. Annu Rev Cell Dev Biol 11:35–71
30. To LB, Haylock DN, Simmons PJ, Juttner CA (1997) The biology and clinical uses of blood stem cells. Blood 89:2233–2258
31. Link DC (2000) Mechanisms of granulocyte colony-stimulating factor-induced hematopoietic progenitor-cell mobilization. Semin Hematol 37:25–32
32. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S (2001) Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 7:430–436

33. Wright DE, Bowman EP, Wagers AJ, Butcher EC, Weissman IL (2002) Hematopoietic stem cells are uniquely selective in their migratory response to chemokines. J Exp Med 195:1145–1154
34. Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, Ponomaryov T, Taichman RS, Arenzana-Seisdedos F, Fujii N, Sandbank J, Zipori D, Lapidot T (2002) G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol 3:687–694
35. Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS (2003) Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 362:697–703
36. Norol F, Merlet P, Isnard R, Sebillon P, Bonnet N, Cailliot C, Carrion C, Ribeiro M, Charlotte F, Pradeau P, Mayol JF, Peinnequin A, Drouet M, Safsafi K, Vernant JP, Herodin F (2003) Influence of mobilized stem cells on myocardial infarct repair in a nonhuman primate model. Blood 102:4361–4368
37. Deten A, Volz HC, Clamors S, Leiblein S, Briest W, Marx G, Zimmer HG (2005) Hematopoietic stem cells do not repair the infarcted mouse heart. Cardiovasc Res 65:52–63
38. Camici PG (2004) Hibernation and heart failure. Heart 90:141–143
39. Heusch G, Schulz R, Rahimtoola SH (2005) Myocardial hibernation: a delicate balance. Am J Physiol Heart Circ Physiol 288:H984–H999
40. Wijns W, Vatner SF, Camici PG (1998) Hibernating myocardium. N Engl J Med 339:173–181
41. Hasegawa H, Takano H, Iwanaga K, Ohtsuka M, Qin Y, Niitsuma Y, Ueda K, Toyoda T, Tadokoro H, Komuro I (2006) Cardioprotective effects of granulocyte colony-stimulating factor in swine with chronic myocardial ischemia. J Am Coll Cardiol (in press)
42. Podewski EK, Hilfiker-Kleiner D, Hilfiker A, Morawietz H, Lichtenberg A, Wollert KC, Drexler H (2003) Alterations in Janus kinase (JAK)-signal transducers and activators of transcription (STAT) signaling in patients with end-stage dilated cardiomyopathy. Circulation 107:798–802
43. Osugi T, Oshima Y, Fujio Y, Funamoto M, Yamashita A, Negoro S, Kunisada K, Izumi M, Nakaoka Y, Hirota H, Okabe M, Yamauchi-Takihara K, Kawase I, Kishimoto T (2002) Cardiac-specific activation of signal transducer and activator of transcription 3 promotes vascular formation in the heart. J Biol Chem 277:6676–6681
44. Oshima Y, Fujio Y, Nakanishi T, Itoh N, Yamamoto Y, Negoro S, Tanaka K, Kishimoto T, Kawase I, Azuma J (2005) STAT3 mediates cardioprotection against ischemia/reperfusion injury through metallothionein induction in the heart. Cardiovasc Res 65:428–435
45. Wallace KB (2003) Doxorubicin-induced cardiac mitochondrionopathy. Pharmacol Toxicol 93:105–115
46. Kunisada K, Negoro S, Tone E, Funamoto M, Osugi T, Yamada S, Okabe M, Kishimoto T, Yamauchi-Takihara K (2000) Signal transducer and activator of transcription 3 in the heart transduces not only a hypertrophic signal but a protective signal against doxorubicin-induced cardiomyopathy. Proc Natl Acad Sci USA 97:315–319
47. Zou Y, Takano H, Mizukami M, Akazawa H, Qin Y, Toko H, Sakamoto M, Minamino T, Nagai T, Komuro I (2003) Leukemia inhibitory factor enhances survival of cardiomyocytes and induces regeneration of myocardium after myocardial infarction. Circulation 108:748–753
48. Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, Kim YJ, Soo Lee D, Sohn DW, Han KS, Oh BH, Lee MM, Park YB (2004) Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet 363:751–756
49. Kong D, Melo LG, Gnecchi M, Zhang L, Mostoslavsky G, Liew CC, Pratt RE, Dzau VJ (2004) Cytokine-induced mobilization of circulating endothelial progenitor cells enhances repair of injured arteries. Circulation 110:2039–2046
50. Valgimigli M, Rigolin GM, Cittanti C, Malagutti P, Curello S, Percoco G, Bugli AM, Porta MD, Bragotti LZ, Ansani L, Mauro E, Lanfranchi A, Giganti M, Feggi L, Castoldi G, Ferrari R (2005) Use of granulocyte-colony stimulating factor during acute myocardial infarction to enhance bone marrow stem cell mobilization in humans: clinical and angiographic safety profile. Eur Heart J 26:1838–1845
51. Kuethe F, Figulla HR, Herzau M, Voth M, Fritzenwanger M, Opfermann T, Pachmann K, Krack A, Sayer HG, Gottschild D, Werner GS (2005) Treatment with granulocyte colony-stimulating factor for mobilization of bone marrow cells in patients with acute myocardial infarction. Am Heart J 150:115 (e1–7)
52. Jorgensen E, Ripa RS, Helqvist S, Wang Y, Johnsen HE, Grande P, Kastrup J (2006) In-stent neo-intimal hyperplasia after stem cell mobilization by granulocyte-colony stimulating factor preliminary intracoronary ultrasound results from a double-blind randomized placebo-controlled study of patients treated with percutaneous coronary intervention for ST-elevation myocardial infarction (STEMMI Trial). Int J Cardiol (in press)

Serum Tenascin-C might be a Novel Predictor of Left Ventricular Remodeling and Prognosis after Acute Myocardial Infarction.

Akira Sato, MD,* Kazutaka Aonuma, MD,† Kyoko Imanaka-Yoshida, MD,‡ Toshimichi Yoshida, MD,‡ Mitsuaki Isobe, MD,§ Daisuke Kawase,¶ Noriaki Kinoshita, PhD,¶ Yoshio Yazaki, MD,# and Michiaki Hiroe, MD#

*Department of Cardiology, Yokosuka Kyosai General Hospital, Yokosuka, Japan

†Department of Cardiology, University of Tsukuba Graduate School of Comprehensive Human Science, Tsukuba, Japan

‡The Departments of Pathology and Matrix Biology, Mie University Graduate School of Medicine, Tsu, Japan

§Department of Cardiovascular Medicine, Tokyo Medical and Dental Postgraduate School of Medicine, Tokyo, Japan

¶IBL Co. Ltd, Gunma, Japan

#Department of Nephrology and Cardiology, International Medical Center of Japan, Tokyo, Japan

Short title: Tenascin-C after myocardial infarction

Address correspondence to:

Kyoko Imanaka-Yoshida

Department of Pathology and Matrix Biology, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, JAPAN

Tel: +81-59-231-5009

Fax: +81-59-231-5210

E-mail: imanaka@doc.medic.mie-u.ac.jp

Total word count: 4547

Abstract

Objective: We investigated clinical implications of serum Tenascin C (TN-C) levels in patients with acute myocardial infarction (AMI).

Background: TN-C, an extracellular matrix glycoprotein, is not normally expressed in the adult heart, but transiently appears during pathological conditions and plays important roles in tissue remodeling.

Methods: Serum TN-C levels were measured by ELISA in 105 AMI patients at various time points, in 10 old MI patients, and 20 normal controls.

Results: The mean serum TN-C level of AMI patients on admission (63.3 ± 30.1 ng/ml) was significantly higher than that of controls and old MI (30.9± 8.8 and 27.4± 11.7 ng/ml, respectively, $p<0.01$), and peaked at 5 days (83.2±43.0 ng/ml). Follow-up examination (mean: 43.9±19.6 months) revealed that 25 of 105 AMI (23.8 %) patients showed left ventricular (LV) remodeling (≥ 20% end-diastolic volume increase), and in 15 (14.3 %), major adverse cardiac events (MACE) were detected. The peak TN-C level was significantly higher in the remodeling group than the non-remodeling group (112 ± 37 versus 66 ± 29 ng/ml; $p < 0.0001$). By receiver-operating characteristic (ROC) analysis, TN-C levels clearly discriminated prediction of LV remodeling and MACE compared with other variables including plasma BNP, CK-MB, and LV function. Best predictive values of TN-C for remodeling and MACE were 84.8 and 92.8 ng/ml, respectively. Cox proportional hazards model analysis showed that TN-C was an important independent predictor of MACE.

Conclusion: The findings suggest that serum TN-C levels might be useful in predicting LV remodeling after AMI.

Key words: infarction; remodeling ; tenascin; inflammation; extracellular matrix; ELISA

Condensed Abstract:

Tenascin-C (TN-C), an extracellular matrix glycoprotein, transiently appears in the pathologic heart, playing important roles in tissue remodeling. We measured serum TN-C levels in 105 acute myocardial infarction (AMI) patients using ELISA. Serum TN-C levels were significantly elevated during the acute stage after AMI and peaked within 5 days. Peak TN-C levels were significantly higher in the left ventricular (LV) remodeling group than the non-remodeling group. AMI patients with high TN-C levels were at much higher risk of MACE. Thus, serum TN-C levels in acute stages following AMI might be a predictive biomarker of LV remodeling and prognosis.

Abbreviations:

AMI	Acute Myocardial Infarction
OMI	Old Myocardial Infarction
ELISA	Enzyme-Linked Immunosorbent Assay
LVEDV	Left Ventricular End-diastolic Volume
LVESV	Left Ventricular End-systolic Volume
LV	Left Ventricle
TDS	Total Defect Score
ROC	Receiver-Operating Characteristic
MACE	Major Cardiac Adverse Events
SPECT	single photon emission computed tomography

Introduction

Left ventricular (LV) remodeling following acute myocardial infarction (AMI) is a major predictor of morbidity and mortality for overt congestive heart failure (CHF) and life-threatening arrhythmias (1). It occurs in an appreciable proportion of patients with AMI successfully treated with primary PTCA despite sustained patency of the infarct-related artery and preservation of regional and global LV functions (2). Therefore, it may be important to identify patients at risk of LV remodeling to prevent LV dilation after AMI. It has been reported that infarct size, anterior infarct location, perfusion status of the culprit lesion, and CHF on admission are major predictors of LV dilatation. Recently, several factors including B-type natriuretic peptide (BNP), cardiac troponin I (cTnI), and high sensitivity C-reactive protein (hsCRP) have been examined as potential predicting biomarkers of LV remodeling (2).

Tenascin-C (TN-C) is an extracellular matrix protein specifically expressed at high levels during embryonic development, wound healing, and cancer invasion, and involved in regulation of cell behavior during tissue remodeling in various tissues (3-6). In the heart, TN-C is normally expressed in early-stage embryos, playing important roles in development of the myocardium, valves, and coronary vessels; but is not detected in adults (7). However, it is re-expressed under pathological conditions such as AMI (8,9), hibernation (10), and myocarditis (11-13), and is closely associated with tissue injury and inflammation. Based on these specific expression patterns, we recently revealed that immunostaining of myocardial tissues (11,13) and immunoscintigraphic imaging (12) for TN-C could be useful in diagnosis of active myocarditis. Furthermore, using an experimental model of myocardial infarction, we found that TN-C transiently appeared during acute stages, with several significant roles in myocardial tissue

remodeling (8,14). Therefore, we hypothesized that TN-C expression levels might be useful for the diagnosis and determination of LV remodeling following AMI. In this study, to clarify clinical implications of TN-C levels in patients with AMI, we assessed serum TN-C concentrations with reference to cardiac function and patient outcomes.

Methods

Study Population

We prospectively studied 105 patients with AMI (73 males and 32 females; mean age, 66 ± 12 years old) admitted to Yokosuka Kyosai Hospital between January 2000 and March 2003, 10 patients with old MI (OMI; 8 males and 2 females; mean age, 66 ± 9 years old), and 20 normal volunteers (14 males and 6 females; mean age, 49 ± 15 years old). Inclusion criteria for this study for AMI patients were as follows: 1) chest pain > 30 min in duration and present within 12 h after onset of symptoms; 2) ST-segment elevation > 0.1 mV with 2 contiguous electocardiographic leads; 3) total occlusion of the infarct-related artery; 4) elevated creatine kinase-MB isoenzymes within 12 h of chest pain; and 5) successful primary coronary angioplasty (defined as Thrombolysis in Myocardial Infarction (TIMI) flow grade 3 (15), and residual diameter stenosis < 30 %). Clinical characteristics of the AMI patients on hospital admission are described in Table 1. The drugs listed in Table 2 were administered at the time of admission according to the discretion of the treating physicians. Written informed consent was obtained from all patients and volunteers, and the study protocol was approved by our institutional review board.

Assay of serum TN-C levels by ELISA.

Blood samples were centrifuged at 15,000 ×g for 15 min, and resulting supernatants were stored at −80 °C until analysis. Serum levels of TN-C with the large subunit containing the C domain of FNIII repeats were determined using an ELISA kit with two monoclonal antibodies, 4F10TT and 19C4MS (IBL, Gunma, Japan), as previously described (16).

Biochemical Analyses

Serum CK-MB levels were analyzed by enzymatic means and plasma BNP concentrations were measured using a specific immunoradiometric commercial assay kit (Shionogi Inc., Japan).

Radionuclide Imaging

ECG gated myocardial SPECT with ^{99m}Tc-tetrofosmin was performed on admission and 6 months later. Imaging was performed at rest in the supine position 1 h after intravenous injection of 740 MBq ^{99m}Tc-tetrofosmin as the radiotracer at both time points using a double-detector SPECT system (PICKER PRISM 2000 XP) equipped with a low-energy high-resolution collimator. Seventy-two projection data were obtained with a 64 × 64 matrix over 360°. Data were acquired for 40 s for each projection. The total acquisition time was approximately 24 min. Images were gated at 16 frames per cardiac cycle with an R-wave trigger and standard parameters similar to the left ventricular ejection fraction (LVEF), LV end-diastolic volume (EDV), and LV end-systolic volume (ESV), which are commercially available with Germano software (17). SPECT images of the LV were divided into 17 segments according to the AHA/ACC recommendations (18). Short-axis slices were separated into eight segments

at the basal and mid-ventricular levels, and the apical portion of one segment was evaluated using vertical long-axis slices. Each segment was visually scored according to 4 grades (0 = normal uptake, 1 = mildly decreased uptake, 2 = moderately decreased uptake, 3 = severely decreased uptake), and total defect scores (TDS) were calculated by summation.

Definition of LV Remodeling and Monitoring of Clinical Events

LV remodeling was defined as an increase in end-diastolic volume at 6 months after infarction of ≥ 20 % in comparison with that based on measurements in individual patients, according to Bolognese et al. (19). Major cardiac adverse events (MACE) defined as cardiac death, non fatal AMI, and hospitalization for CHF were the primary outcomes for the present analysis. After hospital discharge, all patients on medication were monitored at our outpatient clinic for up to 5.5 years.

Immunohistochemistry of TN-C

Immunostainings of heart tissues obtained from 3 AMI, 3 OMI, and 3 non-cardiac disease autopsy cases were performed as previously described (20) using two anti-TN-C mouse monoclonal antibodies. In brief, after treatment with pepsin for 10 min or heating in an autoclave for antigen retrieval, sections were incubated with antibody clone 4F10TT (1 μg/ml) or 6C6MS (10 μg/ml), and then processed using an LSAB kit (Dako Japan, Kyoto, Japan). 6C6MS antibody recognizes the same FNIII repeat of TN-C as 19C4MS antibody, but gives more intense immunostaining than 19C4MS in paraffin-embedded tissues.

Statistical Analysis

The multivariate analysis included all risk factors with probability values of less than 0.05 in a backward stepwise regression model. Receiver-operating characteristic (ROC) analysis was used to determine optimal cut-off values of clinical variables for predictions of LV remodeling and MACE. The ROC curve represents relationships between sensitivity and specificity by plotting true-positive rates against false-positive rates as the cut-off level of the model varies. The area under the ROC curve (AUC) provides a measure of overall accuracy that is independent of decision criterion. The best cut-off value was defined as the point with the highest sum of sensitivity and specificity. Evaluation of statistical differences between groups was determined using Kruskal-Wallis analysis and the Mann-Whitney U-test. Correlations were estimated using Spearman's rank correlation test. Event-free survival curves for MACE were constructed using the Kaplan-Meier method, and statistical differences between curves were assessed using the log-rank test. P values less than 0.05 were considered significant.

RESULTS

Expression of TN-C in Human Myocardium following Myocardial Infarction

Positive immunostainings with the two monoclonal antibodies, 6C6MS and 4F10TT, were observed in infarct lesions of myocardia in AMI patients. In contrast, scar tissue in OMI patients and normal myocardia were negative with both antibodies (Fig.1).

Sequential Changes in Serum TN-C Concentrations following AMI