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G-CSF有助於急性心肌梗塞之治療效果

G-CSF therapy for acute myocardial infarction

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Granulocyte-colony-stimulating factor (G-CSF) has recently been shown to have various effects besides promoting the proliferation and differentiation of myeloid progenitor cells, including the mobilization of bone marrow stem cells and the regeneration infarcted hearts in mice. Recent animal studies have also revealed that G-CSF activates multiple signaling pathways, such as Akt and also the Janus family kinase-2 and signal transducer and activation of transcription-3 (Jak2–STAT3) pathway, in cardiac myocytes. It prevents left ventricular remodeling after myocardial infarction by decreasing cardiomyocyte death and by increasing the number of blood vessels, suggesting the importance of direct actions of G-CSF on the myocardium rather than through mobilization and differentiation of stem cells. Several clinical trials have been performed to study the efficacy of G-CSF therapy in patients with acute myocardial infarction but the results remain controversial because the protocols followed varied between the trials.

Introduction

The number of patients with heart failure has increased over the past 40 years [1]. Although treatments for heart failure have progressed, the prognosis of heart failure is still poor. Conventional treatments cannot repair the injured myocardium, and there is currently no therapy for patients with terminal heart failure other than transplantation. Therefore, novel strategies to treat heart failure have been awaited, and regeneration therapy has recently attracted much attention.

Complex architectural alterations are induced in both the infarcted and non-infarcted myocardium after acute myocardial infarction (AMI). Dilatation and thinning of the left ventricular (LV) wall are the prominent features in the infarcted region. In addition, LV remodeling (see Glossary) with compensatory dilatation and hypertrophy is induced in the non-infarcted region. Animal studies have suggested that the administration of progenitor cells derived from the bone marrow (BM) improves cardiac function after AMI through myocardial regeneration or neovascularization [2–4]. Hematopoietic cytokines such as granulocyte-colony-stimulating factor (G-CSF) and stem cell factor (SCF) mobilize BM stem cells (BMSCs), in addition to regulating the growth and differentiation of hematopoietic progenitor cells [5]. It was reported that the subcutaneous injection of both G-CSF and SCF improved cardiac function and reduced mortality after AMI in splenectomized mice [6]. Cytokine-mediated mobilization of BMSCs resulted in myocardial regeneration, characterized by dividing cardiac myocytes and the formation of vascular structures 27 days after AMI [6]. In recent studies, however, adult hematopoietic stem cells have been reported not to transdifferentiate into cardiac myocytes in the mouse AMI model [7–9]. Although LV dilatation and dysfunction after AMI were modestly prevented in the cell-treated group, the BM cells transplanted into the ischemic myocardium expressed the hematopoietic marker and myeloid marker but not the cardiac tissue-specific markers [8].

In terms of cytokine treatments, it has been reported that the G-CSF treatment started immediately after AMI (experimentally induced by the permanent ligation of the left

Glossary

Hematopoietic stem cells: stem cells and the early precursor cells giving rise to all blood cell types, including erythroid, myeloid and lymphoid lineages. Pretreatment with hematopoietic cytokines such as G-CSF induces the mobilization of hematopoietic stem cells from the bone marrow compartment.
In-stent restenosis: restenosis occurring within the stent. Percutaneous transluminal coronary angioplasty is highly effective for patients with coronary artery disease but the high rate of angiographic restenosis associated with this procedure was a problem. The use of stents has reduced the incidence of restenosis; however, there remains a problem of in-stent restenosis.
Ki67: Ki67 is expressed in nuclei in G1, S, G2, prophase and metaphase. Quiescent cells in G0 do not express Ki67 and this protein is not implicated in DNA repair. The expression of Ki67 means that the cell is in the cell cycle.
Left ventricular (LV) remodeling: structural changes in the LV observed in cardiovascular diseases such as hypertension, valvular heart disease, myocarditis, dilated cardiomyopathy and myocardial infarction. The process of LV remodeling is influenced by hemodynamic load, neurohumoral activation and other factors. Although LV remodeling is initially an adaptive response for maintaining normal cardiac function, it often causes congestive heart failure.
Percutaneous coronary intervention (PCI): a revascularization, mainly by balloon inflation and/or stent implantation, in the treatment of coronary artery disease. The major problem of PCI is renarrowing of the dilated vessel after the procedure (restenosis).
Preconditioning: a phenomenon first described in experimental preparations in which brief episodes of ischemia and reperfusion applied prior to a longer coronary artery occlusion reduce myocardial infarct size. It is also known in the clinical setting that preinfarct angina reduces infarct size and is associated with a better clinical outcome.
Stenosis score: the degree of stenosis in the coronary arteries. The stenosis score is graded into 10 points (scale of 0–9) and the points are summed for each section.
Watanabe heritable hyperlipidemic rabbits: an animal model for human familial hypercholesterolemia and atherosclerosis. This strain of the rabbit is characterized by a genetic deficiency or mutation of functional low-density lipoprotein receptors, and the rabbit develops severe atherosclerosis, which is pathologically similar to human familial homozygous hyperlipidemia.

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coronary artery) is as effective as the treatment started before AMI, and that the treatment with G-CSF alone also has beneficial effects, to a similar degree to the combination treatment of G-CSF and SCF in the non-splenectomized mouse model [10]. The number of apoptotic cells was decreased in the border area of the G-CSF-treated hearts after AMI. Although many BM-derived cells were recognized in the border area of the treatment group but not the control group, most of the BM-derived cells were infiltrated blood cells and some cells were endothelial cells (ECs) [10]. The number of capillaries in the border area after AMI was far greater in the treatment group than in the control group. The timing of starting G-CSF treatment is important. The beneficial effects of G-CSF were significantly larger when the G-CSF treatment was started earlier after AMI [11]. The treatment that started at 3 days after AMI was less effective than that started immediately after AMI, and the treatment started at 7 days after AMI had almost no effects [11].

Many groups have reported the beneficial effects of G-CSF on the prevention of LV remodeling and dysfunction after AMI in various animal models [12–14]. In clinical trials, however, the efficacy of G-CSF therapy in patients with AMI is controversial. Here, we summarize the cardioprotective effects and mechanisms of G-CSF action and discuss the controversies generated among clinical trials.

Molecular mechanisms of G-CSF-induced cardioprotective effects

The expression of the G-CSF receptor mRNA and protein has been detected in cardiomyocytes of mice and rats [11]. G-CSF significantly activated Janus kinase-2 (Jak2), signal transducer and activator of transcription (STAT)1 and STAT3 in cardiomyocytes in a dose-dependent manner [11]. Many cardiomyocytes die immediately after AMI, and G-CSF prevents cardiomyocyte death [10,11,15]. Expression levels of antiapoptotic proteins such as Bcl-2 and Bcl-xL, which are target molecules of the Jak–STAT pathway, were decreased in cultured cardiomyocytes after the addition of hydrogen peroxide, and the change was attenuated by pretreatment with G-CSF. A dominant negative STAT3 was used, in which tyrosine residue 705 is mutated to phenylalanine, to examine the role of STAT3 in G-GCF action [11]. Overexpression of dominant negative STAT3 (dnSTAT3) in cardiomyocytes using adenovirus inhibited the protective effects of G-CSF [11]. Animal studies have demonstrated that STAT3 protects the heart against pathophysiological stresses such as myocardial ischemia, mechanical stress and cytotoxic agents [16–18]. Although there were no significant differences in LV function and size between wild-type (Wt) and transgenic mice with cardiac-specific overexpression of dnSTAT3 (dnSTAT3-Tg) at the basal level, the cardioprotective effects of G-CSF on post-AMI hearts were abolished in dnSTAT3-Tg mice [11], suggesting that G-CSF has cardioprotective effects through the Jak2–STAT3 pathway in cardiomyocytes [11,19]. G-CSF increased the number of hematopoietic stem cells in peripheral blood both in Wt and dnSTAT3-Tg mice but did not increase the cardiac engraftment of BM cells in either group. Furthermore, G-CSF did not affect the number of cardiac stem cells in the post-AMI hearts of both Wt and dnSTAT3-Tg mice. The number of Ki67-positive cardiomyocytes in the hearts was increased after AMI but G-CSF did not alter the cell number in the hearts of either Wt or dnSTAT3-Tg mice [11]. These results suggest that G-CSF does not induce the proliferation of cardiomyocytes. Taken together, the beneficial effects of G-CSF on infarcted hearts can be attributable to its direct protective action on the myocardium rather than to the mobilization, differentiation and proliferation of stem cells.

It has been also reported that G-CSF attenuates early ventricular dilatation after AMI in rats [12]. Expression levels of transforming growth factor-β and procollagen mRNA in the infarcted area at 3 days were higher in the G-CSF group than in the control group. The accumulation of collagen fibers can reinforce the myocardium by increasing stress tolerance and limiting ventricular expansion. G-CSF prevented cardiac remodeling and dysfunction at 3 months in the ischemia–reperfusion rabbit model [13]. G-CSF increased the number of macrophages in the infarcted area at 2 days after AMI, and the expression levels of matrix metalloproteinase (MMP)-1 and MMP-9 in the ischemic region at 7 days after AMI. Rapid absorption of necrotic tissues by macrophages at the acute stage might contribute to the beneficial effects on the hearts through acceleration of the healing process. Because the excessive extent of fibrosis accelerates cardiac remodeling and decreases cardiac function, an increase in levels of MMPs might protect against cardiac fibrosis through proteolysis of excessive collagen at the subacute stage. G-CSF induced non-hematopoietic mesenchymal stem cells in the BM to mobilize into the infarcted area and differentiate into cardiomyocytes after AMI in mice [14].

G-CSF also has acute protective effects, even on the ischemia–reperfusion heart [20]. G-CSF markedly reduced infarct size after ischemia–reperfusion in the isolated perfused heart. G-CSF-induced reduction of infarct size was abolished by inhibitors of phosphatidylinositol 3-kinase, Jak2 and nitric oxide synthase (NOS) but not by an inhibitor of mitogen-activated protein kinase kinase [20]. Transcriptional regulation by the Jak2–STAT3 pathway is one of the key mechanisms in G-CSF-mediated cardioprotection against the infarcted heart in the late phase, and the Akt–NOS pathway might also contribute to the beneficial effects of G-CSF in the early phase. NOS is phosphorylated and activated by Akt, and NOS-producing NO has been reported to have a pivotal role in the cardioprotection of preconditioning [21]. Although further studies are needed to clarify the factors downstream of NO giving rise to the postconditioning-like effects of G-CSF after ischemia–reperfusion injury, these results suggest that the direct action on the myocardium is important for the cardioprotective effects of G-CSF (Figure 1).

Preclinical studies have demonstrated that G-CSF treatment improves cardiac dysfunction and reduces LV remodeling after AMI, even in large animals such as swine and dogs [22,23]. In the ischemic region, there were fewer apoptotic ECs and more vessels in the G-CSF group than in the control group. Moreover, vascular endothelial growth factor was more abundantly expressed and Akt was more strongly activated in the ischemic region in the G-CSF group than in the control group [22,23].

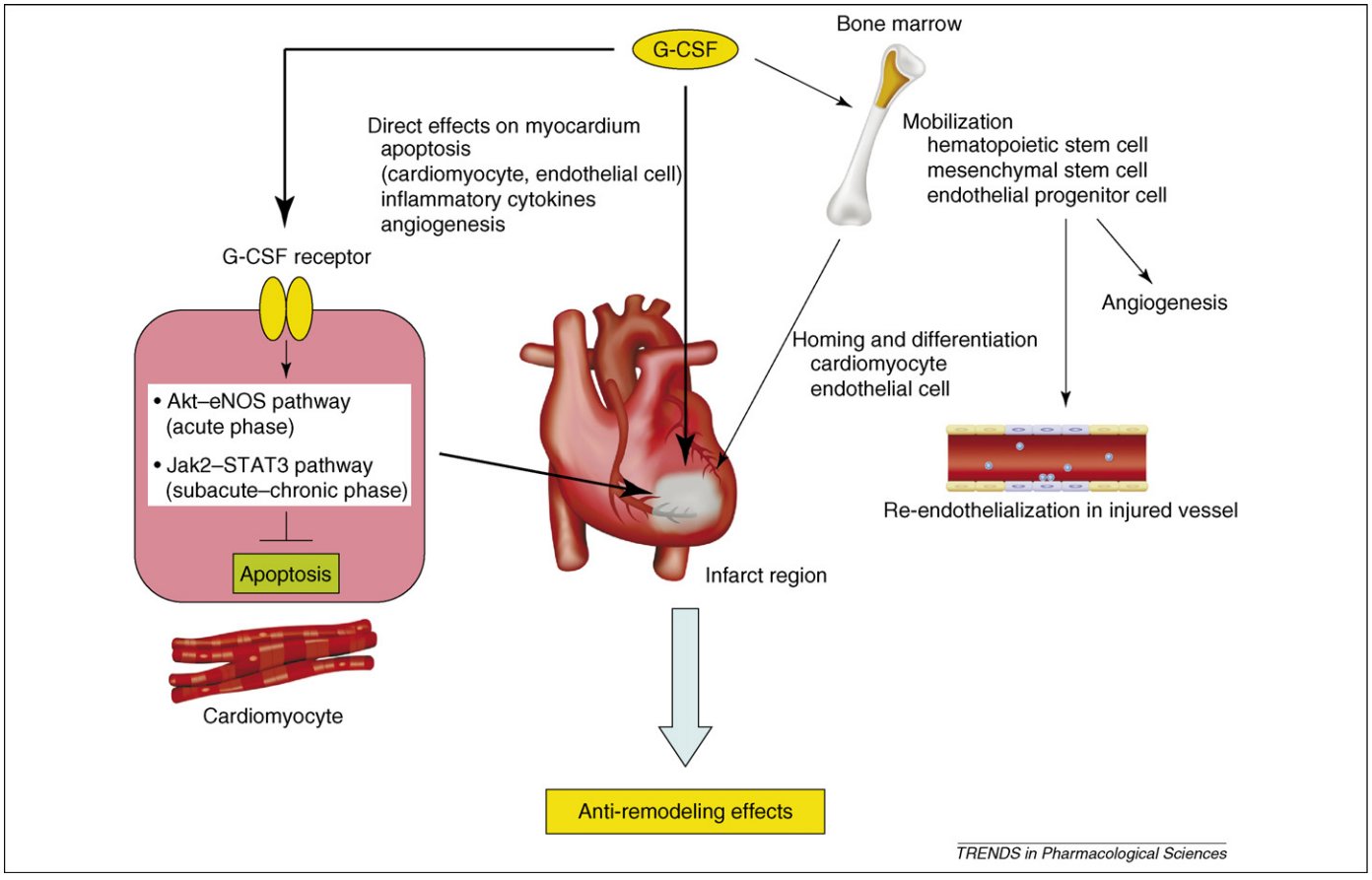

Figure 1. Hypothetical scheme demonstrating the mechanisms of cardioprotection induced by G-CSF. The mechanisms shown by the thicker lines are suggested as being important.

Transcriptional regulation by the Jak2–STAT3 pathway is one of the key mechanisms in G-CSF-mediated cardioprotection against the infarcted heart in the chronic stage, whereas the Akt–endothelial NOS (eNOS) pathway might be important in the acute stage. eNOS has been reported to be phosphorylated by Akt, and activated eNOS-producing NO has been reported to have a pivotal role in the cardioprotection of preconditioning [21].

Clinical trials

Based on the experimental results in animal models, a clinical trial evaluating the feasibility and safety of G-CSF in patients with AMI has been carried out [24]. Patients with AMI or old MI who were subjected to subcutaneous injections of G-CSF (10 $\mu g\,kg^{-1}$) for 4 days before percutaneous coronary intervention (PCI) were unexpectedly reported to have high rates of in-stent restenosis [24]. In the study, patients did not receive primary PCI during the 'golden time' of AMI treatment (<12 hours after onset of AMI). Only a few patients were assessed by coronary angiography at six-month follow-up. In a non-randomized study [25], G-CSF (10 $\mu g\,kg^{-1}$) treatment for 5 days induced serious adverse events in high-risk patients with severe coronary artery disease. Because all 16 patients had Canadian Cardiovascular Society (CCS) functional class 3 or 4 angina despite prior revascularization, there is a possibility that these patients had many destabilized plaques in their coronary arteries [25].

To elucidate the safety of G-CSF therapy in patients with coronary artery disease, the effect of G-CSF on atherosclerosis was investigated. Treatment with G-CSF significantly reduced the stenosis score of the coronary artery and the lipid plaque area of thoracic aorta in Watanabe heritable hyperlipidemic rabbits [26]. G-CSF treatment also accelerated re-endothelialization and inhibited neointimal thickening after vascular injury in mice, rats and rabbits [26–30]. In fact, the safety of G-CSF treatment for patients with coronary artery disease such as AMI and angina pectoris has been proven by recent clinical trials, as mentioned later.

The effects of G-CSF (10 $\mu g\,kg^{-1}$ for 7 days) on patients with AMI who were treated with PCI were examined in a prospective, non-randomized study [31]. Single-photon emission computed tomography (SPECT) imaging demonstrated that the G-CSF group ($n = 14$) significantly improved the regional wall motion, wall perfusion and LV ejection fraction (LVEF) compared with the control group ($n = 9$) at 3 months after AMI. No severe adverse effects were observed in the G-CSF group [31]. The effects of G-CSF (5 $\mu g\,kg^{-1}$ for 4 days) in patients with AMI who were subjected to PCI with stenting were examined in a randomized trial (Table 1) [32]. Quantitative gated SPECT analysis showed that the relative increases in LVEF and LV end-diastolic volume tended to be higher and lower, respectively, in the G-CSF group ($n = 8$) compared with the control group ($n = 8$) at 6 months' follow-up, although these

Table 1. Summary of randomized trials evaluating G-CSF therapy in AMI

G-CSF (dose × duration)	Mean AMI to G-CSF (PCI to G-CSF)	Number of patients	Outcomes (G-CSF vs. control)			Study name and Refs
			Time and method	LVEF	LV size	
5 μg kg^{-1} × 4 days	37 h (NM)	G-CSF (n = 10); control (n = 10)	6 months (SPECT)	Unchanged (P = 0.068);	LV size unchanged (P = 0.054)	[32]
10 μg kg^{-1} × 6 days	6.5 h (1.5 h)	G-CSF (n = 25); control (n = 25)	4 months (LVG, UCG, PET)	↑ (P < 0.001)	LV size ↓ (P < 0.002)	FIRSTLINE-AMI [33]
10 μg kg^{-1} × 5 days	5 days (NM)	G-CSF (n = 56); control (n = 58)	4–6 months (SPECT, MRI)	Unchanged (P = 0.14)	Infarct size unchanged (P = 0.56)	REVIVAL-2 [35]
10 μg kg^{-1} × 6 days	33.5 h[b] (29.5 h[b])	G-CSF (n = 39); control (n = 39)	6 months (MRI)	Unchanged (P = 0.9)	LV size unchanged (P = 0.7)	STEMMI [39]
2.5 μg kg^{-1} × 5 days	21 h (14.5 h)	G-CSF (n = 18); control (n = 22)	6 months (SPECT)	↑ (P = 0.013)	LV size ↓ (P = 0.007)	GLEAM [40]
10 μg kg^{-1} × 5 days	63 h (31 h)	G-CSF (n = 23); control (n = 21)	3 months (MRI)	Unchanged (P = 0.770)	LV size unchanged (NS)	G-CSF-STEMI [41]

[a]Abbreviations: NM, not mentioned; NS, not significant.
[b]Median.

differences were not statistically significant [32]. At follow-up, the two groups did not differ in the rate of coronary restenosis. The Front Integrated Revascularization and Stem cell Liberation in Evolving Acute Myocardial Infarction (FIRSTLINE-AMI) study analyzed the effects of G-CSF (10 μg kg^{-1} for 6 days) in a randomized study on patients with AMI who were subjected to PCI with stenting [33,34]. G-CSF administration was started 1.5 hours after reperfusion. Echocardiographic analysis demonstrated that G-CSF treatment significantly improved LVEF and wall motion score index at 4 months. LV end-diastolic diameter showed no change in the G-CSF group (n = 25), whereas the LV end-diastolic diameter progressively enlarged in the control group (n = 25) and there was worsening of LVEF at 4 months compared with baseline in the control group. There was no difference in restenosis rate between the two groups.

By contrast, the Regenerate Vital Myocardium by Vigorous Activation of Bone Marrow Stem Cells (REVIVAL-2) randomized trial assessed the effects of G-CSF treatment (10 μg kg^{-1} for 5 days) on a larger number of patients with AMI who had undergone successful reperfusion by PCI [35]. G-CSF administration was started 5 days after symptom onset. The authors' rationale for determining the starting time of the G-CSF treatment was based on clinical data showing that the mobilization of stem cells and endothelial progenitor cells occurs naturally in patients with AMI, peaking at day 7 [36,37]. In fact, the beneficial effects of BM cell infusion on the recovery of LV function were confined to those patients who had been treated for more than 4 days after PCI in the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial [38]. In REVIVAL-2, SPECT and magnetic resonance imaging (MRI) were performed at baseline and at 4–6 months after AMI. Although G-CSF treatment for patients with AMI was safe, there were no significant differences in the changes in infarct size and LVEF between the G-CSF group (n = 56) and the control group (n = 58) [35]. It was also reported in the Stem Cells in Myocardial Infarction (STEMMI) randomized trial that G-CSF treatment (10 μg kg^{-1} for 6 days) was safe but did not lead to further improvement in LV function after AMI [39]. LVEF improved to a similar degree in the G-CSF group (n = 37) and the control group (n = 33), measured by both MRI and echocardiography from baseline to 6 months. In this study, G-CSF injection was started 33.5 hours (median) after symptom onset. The rate of left anterior descending coronary artery (LAD)-related AMI was higher and the rate of Thrombosis in Myocardial Infarction (TIMI) flow grade 3 was lower in patients in the G-CSF-treated group compared with those in the control group. The change in LVEF from baseline to 6 months was large, even in the control group (8.0%) [39]. Treatment with G-CSF was safe and well tolerated. The feasibility and safety of G-CSF treatment (2.5 μg kg^{-1} for 5 days) was recently evaluated in 40 patients with AMI in a randomized trial (Feasibility and safety of G-CSF Treatment for Left Ventricular Dysfunction After Acute Myocardial Infarction [GLEAM]) [40]. In contrast to the previous trials, this study selected AMI patients with total occlusion of LAD alone because SPECT is not able to assess ischemia accurately when there are stenoses in multiple coronary arteries. All patients achieved TIMI flow grade 3 reperfusion after PCI. Furthermore, to minimize its adverse effects, a low dose of G-CSF (2.5 μg kg^{-1}) was used to prevent a marked increase in white blood cell count. The results demonstrated that LVEF at 6 months was significantly better than that at 4 days in the G-CSF group but was unchanged in the control group. Although no significant difference was observed for LV end-diastolic volume between the two groups, LV end-systolic volume tended to be decreased only in the G-CSF group. In the G-CSF group, the defect score was significantly decreased from 4 days to 6 months after AMI. The restenosis rate at 6 months after AMI was not significantly different between the two groups [40]. The G-CSF-STEMMI trial examined the effects of G-CSF treatment (10 μg kg^{-1} for 5 days) on myocardial function and perfusion in AMI patients undergoing delayed PCI [41]. The time from onset of pain to PCI was >6 hours and <7 days. Global myocardial function from baseline to 3 months improved in both groups but the G-CSF group (n = 23) was not superior to the control group (n = 21). G-CSF resulted in a significant improvement in myocardial perfusion one week and one month after PCI. Occurrence of restenosis was not significantly different between the G-CSF group and the control group [41].

In these studies, G-CSF did not accelerate the rate of restenosis but the efficacy of G-CSF therapy for patients with AMI was inconsistent. There were some differences in the kind of infarct-related artery, TIMI flow grade and the time from AMI to G-CSF administration. The frequency of

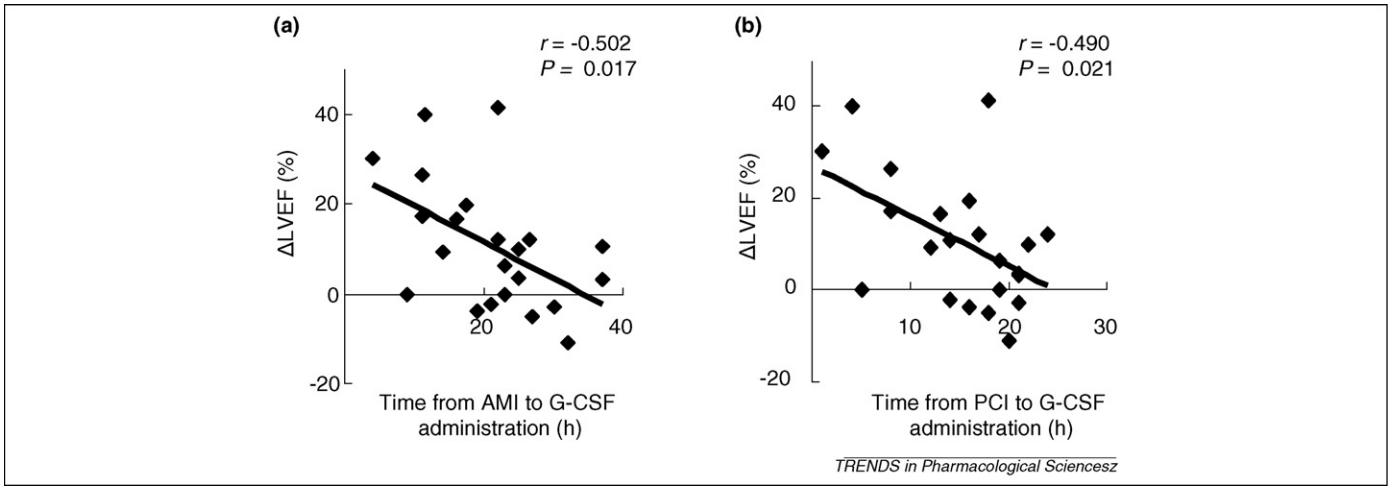

Figure 2. The inverse correlations **(a)** between the time from AMI to G-CSF administration and ΔLVEF and **(b)** between the time from PCI to G-CSF administration and ΔLVEF. Statistical analysis was performed using SPSS software, version 11.5 (SPSS Inc, Chicago, IL, USA). Data from Takano, H. and Komuro, I. (unpublished).

LAD-related AMI was equal between the G-CSF-treated group and the control group, and TIMI flow grade 3 was documented in all patients after PCI in the trials that showed positive results [33,34,40]. Many investigators were of the opinion that G-CSF inhibits LV remodeling and dysfunction after AMI by accelerating cardiac regeneration in the infarcted hearts and did not pay attention to the timing of G-CSF administration. The trials in which G-CSF treatment was started early after AMI seemed to show positive results [33,34,40], whereas the trials in which G-CSF treatment was started late after AMI showed negative results [35,39,41]. These findings suggest that the time interval between AMI and PCI and/or G-CSF treatment causes the difference in outcome (Table 1). Our clinical data demonstrated that there were inverse correlations between the time from AMI to G-CSF administration and the absolute change in LVEF (ΔLVEF), and between the time from PCI to G-CSF administration and ΔLVEF (H.T. *et al*., unpublished) (Figure 2). The beneficial effects of G-CSF were significantly reduced when G-CSF treatment was started late (after 3 days) in the AMI mouse model mentioned earlier [11], and the experimental results are consistent with our clinical data. In most animal studies, the administration of G-CSF was indeed started immediately or within several hours after AMI [10–13,22]. These results suggest that the timing of the treatment could be crucial to obtain the most beneficial effects of G-CSF because the cardioprotective effects of G-CSF might be mainly attributable to a direct action on the myocardium.

Although there is no precise evidence indicating the cross-species bioactivity of recombinant human (rh) G-CSF, a dose of 100 $\mu g\,kg^{-1}\,day^{-1}$ rhG-CSF in mice produces a similar neutrophil response as 10 $\mu g\,kg^{-1}\,day^{-1}$ of rhG-CSF in humans, suggesting that the affinity of rhG-CSF for the murine G-CSF receptor is ~1/10 that for the human G-CSF receptor. The optimal effects of stem cell mobilization in mice are reached with a dose of 250 $\mu g\,kg^{-1}\,day^{-1}$ rhG-CSF or higher, whereas the common dose used in patients is 10 $\mu g\,kg^{-1}\,day^{-1}$, and even 2.5 $\mu g\,kg^{-1}\,day^{-1}$ was effective in the GLEAM study [40], suggesting that 2.5–10 $\mu g\,kg^{-1}\,day^{-1}$ G-CSF has a cardioprotective effect on AMI in humans which is not the result of stem cell mobilization. However, further studies are needed to determine the most appropriate dose of G-CSF in humans.

Concluding remarks

Because the success of novel therapies depends on rigorous basic investigations, considerable attention should be paid to the valuable results emerging from animal experiments and from research into mechanisms of action. When clinical trials are performed to assess the feasibility and safety of novel therapies including cell therapy, gene therapy and cytokine therapy for coronary heart diseases, the inclusion criteria for patients and the appropriate protocol should be strictly determined. The dose of G-CSF that should be used and the stage at which treatment should be started have not yet been determined. In addition, the duration of therapy and the method of administration (e.g. subcutaneous, intravenous or intracoronary) have also yet to be elucidated. It remains unknown which modality is most appropriate to evaluate the effects of G-CSF and when the evaluation should be performed. Further studies, with more rational designs are needed to conclude on the efficacy of G-CSF therapy for AMI.

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