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用G-CSF做幹細胞治療對急性心肌改塞有效

REVIEW

G-CSF for stem cell therapy in acute myocardial infarction: friend or foe?

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Abstract Stem cell-based therapy has emerged as a potential therapeutic option for patients with acute myocardial infarction. The ability of granulocyte colony-stimulating factor (G-CSF) to mobilize endogenous stem cells as well as to protect cardiomyocytes at risk via paracrine effects has attracted considerable attention. In the past decade, a number of clinical trials were carried out to study the efficacy of G-CSF in cardiac repair. These trials showed variable outcomes in terms of improved cardiac contractile function and suppressed left ventricular negative remodelling. Critical examinations of these results have raised doubts concerning the effectiveness of G-CSF in modulating functional recovery. However, these cumulative clinical experiences are helpful in the understanding of mechanisms and roles of signalling pathways in regulating homing and engraftment of bone marrow stem cells to the infarcted heart. In this review, we discuss some of the observations that may have influenced the clinical outcomes. Improving strategies that target the critical aspects of G-CSF-driven cardiac therapy may provide a better platform to augment clinical benefits in future trials.

Keywords G-CSF • Stem cell therapy • Cardiac repair • CXCR4 • Ischaemia • Myocardial infarction

1. Introduction

Clinical presentation of heart failure has increased in the last half century. It is becoming one of the major causes of morbidity in all hospital admissions. It is estimated that about 80.7 million people in the USA suffer from one or more cardiovascular diseases. Hypertension and coronary heart disease constitute a major bulk of these cardiovascular disease cases,[1] wherein myocardial infarction (MI) constitutes half of all coronary heart disease cases. Although there has been substantial advancement in treatments, the prognosis of heart failure is still poor.[2] Currently, acute myocardial infarction (AMI) therapy relies on early coronary reperfusion that alleviates mortality rates, but this conventional therapy cannot reverse the damage to infarcted myocardium.[3] AMI causes complex architectural alterations in the infarcted as well as the non-infarcted regions of the myocardium. Chamber dilatation and left ventricular (LV) wall thinning are the most prominent features post-infarction. This is followed by progressive LV remodelling, which initially acts as an adaptive response, but often leads to congestive heart failure. Furthermore, LV remodelling with compensatory dilatation and hypertrophy is also induced in the non-infarcted regions of the heart.[2]

Evidence of heart regeneration in resected ventricle in zebra fish[4] and application of stem cells in heart repair[5] provided a clear indication that cell-based therapies may provide an exciting opportunity for patients afflicted with MI or ischaemic heart diseases. The concept of cell-based therapies revolves on generation of new myocytes from stem cells to replace damaged myocardial tissues, and their paracrine factors in mediating healing, angiogenesis and cell survival, leading to restoration of cardiac function.[6–8] Bone marrow is the major reservoir of stem cells, and these bone marrow stem cells (BMSCs) are a mixture of haematopoietic progenitor cells, mesenchymal stem cells, and endothelial progenitor cells, that in response to tissue injury are mobilized from bone marrow to the injured site, thus aiding in tissue repair.[9,10] The ability of endothelial progenitor cells to promote angiogenesis in ischaemic tissues,[11,12] and differentiation of mesenchymal stem cells into other lineages such as cardiomyocytes[13] have been postulated to work in combination to help in cardiac repair. These therapeutic properties of stem cells in the context of specific disease treatment have been highly anticipated due to their promising outcomes.[14] However, practical and technical problems associated with harvesting, isolating, expanding, and delivering of these cells have yet to be fully resolved.

In contrast, a strategy to mobilize stem cells has been established clinically with granulocyte colony-stimulating factor (G-CSF).[15] G-CSF, a 25 kDa haematopoietic cytokine,[16] has been used clinically in the treatment of neutropenia and for bone marrow transplantations. Notably higher levels of G-CSF are produced by infarcted heart, making it a potential agent for cardiac repair. Furthermore,

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experimental models with AMI have shown that G-CSF administration significantly mobilizes BMSCs to the heart, which is accompanied by reduced left ventricular remodelling and improved cardiac function.[17,18] These initial studies lend credence to the beneficial role of G-CSF in AMI. Based on these observations, clinical trials were performed and are being carried out in patients with AMI.

In this article, we highlight some possible reasons that may be responsible for the controversial results in previous clinical trials conducted with G-CSF to restore cardiac function. Besides highlighting current practices of G-CSF usage in MI patients, we discuss the major pathways that are crucial in homing and engraftment of cells in the infarcted heart. An insight into these variables would provide valuable information for designing better-controlled trials to extract clinical values of G-CSF in cardiac therapy.

2. Mode of action of G-CSF for cardiac repair

Various mechanisms have been proposed for the beneficial effects of G-CSF in the infarcted heart.[19–21] They include regeneration of myocardium,[9] acceleration of healing process,[22] direct protection of cardiomyocytes from apoptosis,[7] protection of salvaged cardiomyocytes, and reduction of myocardial fibrosis.[23] The ability of G-CSF to translocate BMSCs to the infarcted site has been well documented.[9,24,25] This ability of G-CSF generated keen interest in its use to potentially repair the injured myocardium. A series of small non-randomized clinical trials supported the idea that G-CSF could be of benefit in late treatment of AMI, but the results of these trials have been mixed.[26,27] These studies highlighted the pressing needs in elucidating other associated factors in order to achieve better therapeutic regimes using G-CSF.

2.1 G-CSF and JAK–STAT3 pathway

In their study to understand the mechanism of G-CSF in preventing ventricular remodelling,[7] Harada *et al.* reported expression of G-CSF receptor (G-CSFR) on cardiomyocytes as well as activation of Janus family tyrosine kinase 2 (Jak2) and downstream signalling molecule, signal transducer and activator of transcription 3 (STAT3), in cultured cardiomyocytes by G-CSF. Furthermore, G-CSF enhanced STAT3 activity, increased expression of B-cell lymphoma 2 (Bcl_2) and B-cell lymphoma 2-extra large (Bcl_{XL}) in the infarcted heart, thereby preventing cardiomyocyte apoptosis and cardiac dysfunction (*Figure 1*). These cardioprotective effects of G-CSF were abolished when STAT3 activation was disrupted by AG490, demonstrating a direct cardioprotective action of G-CSF in preventing left ventricular remodelling after myocardial infarction.[7] G-CSF-activated Jaks subsequently phosphorylate the cytoplasmic phosphotyrosine residues in the G-CSFR. Monomeric STATs are in turn phosphorylated on the cytoplasmic portion of the receptor complex. The dimeric STAT then dissociates from the receptor complex and translocates

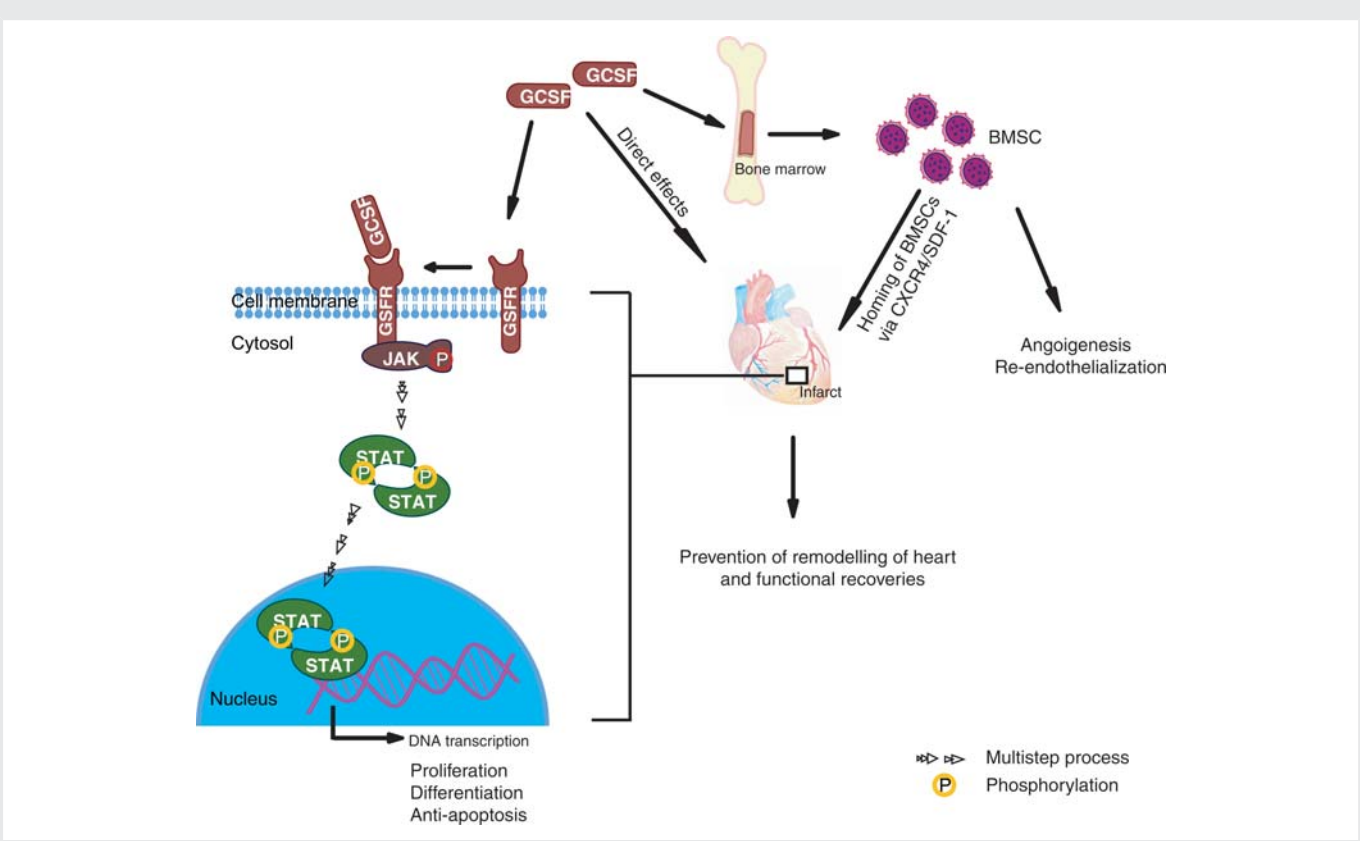

Figure 1 Mode of action of G-CSF in cardiac repair. G-CSF provides a beneficial effect through various modes of action in patients with myocardial infarction. G-CSF induces the migration of bone marrow stem cells (BMSCs), helping in re-endothelialization, angiogenesis and homing in infarcted regions via SDF-1/CXCR4 signalling. Paracrine effects as well as activation of the Jak–STAT3 pathway by G-CSF also help in preventing cardiac remodelling. However, this diagram does not preclude the role of other signalling pathways that are triggered by G-CSF.

to the nucleus, where it binds to specific response elements and induces transcription of angiogenic factors[28] (*Figure 1*). Furthermore, overexpression studies with dominant negative STAT3, in which the 705-tyrosine residue was mutated to phenylalanine in cardiomyocytes inhibited the protective effects of G-CSF, further confirmed its role in cardioprotection.[7] Indeed, the detailed cardioprotective role of the Jak–STAT pathway has been reviewed elsewhere.[29,30]

Apart from activating the Jak–STAT pathway, G-CSF and its receptor are also specifically expressed in embryonic heart at the mid-gestational stage, and expression levels of both molecules is maintained throughout embryogenesis, implicating a role for G-CSF/G-CSFR in cardiogenesis. Furthermore, addition of G-CSF to embryonic stem cells (ESCs) or induced pluripotent stem cell (iPS)-derived cardiomyocytes not only augmented proliferation of cardiomyocytes, but also substantially elevated the expression of the cardiac committed marker, Nkx2.5, further confirming the unique role of G-CSF in cardiogenesis.[16]

2.2 G-CSF and other pathways

The Jak–STAT pathway up-regulates expression of cyclooxygenase-2 and nitric oxide synthase (NOS) 2, and also regulates mitochondrial permeability transition pore inhibition, vascular endothelial growth factor (VEGF; angiogenic and cardioprotective agent), the anti-oxidants manganese superoxide dismutase, metallothioneins (MT1 and MT2), and matrix metalloproteases that are important in repair or scar formation.[29,30] Although the Jak2–STAT3 pathway is the key mechanism in G-CSF-mediated cardioprotection, other pathways, such as Akt–NOS, might also contribute to cardioprotection. Rat hearts subjected to ischaemia followed by reperfusion with G-CSF showed reduction in infarct size along with strong activation of the Akt–NOS pathway. Furthermore, these effects could be abolished with specific inhibitors to NOS, phosphoinositide 3 kinase (PI3K) and Jak2.[31] Moreover, NO expression downstream of the Akt-activated NOS pathway has been reported to have a role in cardioprotection through pre-conditioning of myocytes.[32] However, further studies are needed to clarify the molecules downstream of NO that are involved in the pre-conditioning-like effects of G-CSF after ischaemia–reperfusion injury.

Mitochondria are central to myocardial energetics and cardiac pathophysiology.[20,33] Although only a limited amount is known about the co-relationship between cardiac stress and mitochondrial dysfunction, recent studies have demonstrated that mild stimulation with doxorubicin (Dox) in C57/BL6 mice caused damage to mitochondrial organization, but did not result in cardiac apoptosis, or changes in cardiac systolic function or left ventricular size. Administration of G-CSF improved ATP generation as well as rescuing Dox-impaired mitochondrial electron transport and oxygen consumption.[20] Recently, Carrao *et al.* demonstrated that G-CSF administration in a rat model of repetitive episodic myocardial ischaemia significantly increased coronary collateralization through enhanced production of angiogenic factors. Furthermore, this effect was attributed to an increase in production of reactive oxygen species by cardiomyocytes, rather than neutrophils, and the G-CSF effect was reversible by apocyanin.[34]

2.3 G-CSF and homing of stem cells

Stromal derived factor-1a (SDF-1a) and its receptor chemokine (CXC motif) receptor 4 (CXCR4) have been reported to play important roles in homing of stem cells,[35] embryogenesis and cardiovascular development.[16,36] Furthermore, the SDF-1–CXCR4 homing axis is not restricted to the heart, but is also observed in other cell types.[37–39] The SDF-1–CXCR4 axis is pivotal in retaining stem cells in the bone marrow niche,[40] whereby high expression of SDF-1 in the local hypoxic microenvironment of bone marrow exerts a strong chemotactic effect on CXCR4-expressing stem cells within the niche.[41] However, acute MI with its ensuing apoptosis and ischaemia disrupts such homeostasis by massive up-regulation of SDF-1 in the injured myocardium. This dynamic shift of the SDF-1 axis results in the mobilization, migration and homing of the progenitors or stem cells from bone marrow to the infarcted sites.[42] Consistently, intramyocardial injection of genetically engineered SDF-1 improved myocardial function and mobilized progenitor cells to the heart.[43] Likewise, G-CSF mobilizes stem cells from their bone marrow niche to the peripheral circulation by disrupting the SDF-1–CXCR4 retention axis (*Figure 1*). Furthermore, G-CSF down-regulated SDF-1 and CXCR4 expression in haematopoietic stem cells[40] and increased cleavage of SDF-1 by CD26,[44] resulting in the release of $CXCR4^+$ stem cells into peripheral blood (*Figure 1*). These $CXCR4^+$ cells are then recruited to the injured myocardium, whereby local SDF-1 expression is elevated following MI. The SDF-1–CXCR4 pathway activates a complex signalling cascade that involves calcium efflux, and activation of protein kinase C and PI3K–Akt.[45] Furthermore, blockage of either SDF-1 or CXCR4 resulted in significant reduction in the recruitment of stem cells to the infarcted areas with decreased neovascularization.[46]

2.4 G-CSF stem cell therapy

Stem cell therapy performed to date could be broadly classified into two categories, first where G-CSF is given for 4–6 days post-MI to mobilize endogenous BM cells directly (*Table 1*) and second, where re-infusion of G-CSF mobilizes BM-derived autologous cells by the intracoronary route within a week post-AMI[47] (*Table 2*). The methods employed in most of the stem cell therapy trials in cardiac repair are summarized in *Figure 2*. Animal studies using bone marrow-derived cells have been shown to increase cardiac function and survival.[48] However, only limited trials have shown favourable outcomes,[26,49] while others[50,51] have not been able to reproduce the beneficial outcomes observed in experimental models.

Lack of substantial evidence of new cardiomyocyte generation, cell-independent paracrine-mediated cardiac repair by neovascularization and anti-apoptosis are believed to be responsible for the beneficial outcomes observed in stem cell therapy. However, this explanation of the mixed clinical outcomes is an oversimplification of the multiple variables of physiological, logistical, technical and operational factors that are involved in stem cell therapy.

2.5 G-CSF therapy and age

Differences in the protocol regimes adopted in clinical trials by various groups could be a reason for the variations in the clinical outcomes. Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction (FIRSTLINE-AMI), a randomized trial, included 56 patients with an average age of 50 years. After successful primary percutaneous coronary intervention (PCI), patients received 10 μg/kg body weight G-CSF daily within 85 min (SD 30 min) of PCI over a period of 6 days in addition to the standard care.[26] Based on the variables assessed, the study concluded that G-CSF might contribute to improvement in ventricular function and prevention of ventricular remodelling (*Table 1*). In contrast,

Table 1 Results of clinical trials with direct mobilization of bone marrow cells by G-CSF

Trial	Study design	Patient (control/test)	G-CSF dose (μg/kg/day)	MI to PCI (days)	PCI to G-CSF (days)	Follow-up (months)	Imaging	Outcomes	Reference
Ellis *et al.*	Randomized with placebo controls	6/12 (6 low dose, 6 high dose)	5 (5), $35 \pm 14 \times 10^9$ leukocytes/L $37 \pm 30 \times 10^6$ CD34/L 10 (5), $42 \pm 7.6 \times 10^9$ leukocytes/L $29 \pm 14 \times 10^6$ CD34/L	Low-dose group: 0.2 ± 0.1 High-dose gp: 0.5 ± 0.7	Low-dose group 1.6 ± 0.3 High-dose group: 1.7 ± 0.3	1	Echocardiography	No change in LV function Restenosis: NA	110
G-CSF-STEMI	Randomized, double-blinded, placebo-controlled phaseII study	18/19	10 (5), $42.9 \pm 29.7 \times 10^9$ leukocytes/L $46.1 \pm 33 \times 10^6$ CD34/L	1.3 ± 1.9	1.3 ± 1.0	6	MRI, angiography	No change in LV function, ↑ perfusion, Restenosis:NS	51
FIRSTLINE-AMI	Randomized study	15/15	10 (6), $55 \pm 8 \times 10^9$ leukocytes/L $66 \pm 54 \times 10^6$ CD34/L	0.2 ± 0.1	0.06 ± 0.02	12	Echocardiography, angiography	↑ LV function, LV size: no enlargement, Restenosis: NS	26
Kuethe	Non-randomized, open-label study	5/5	10 (6.6 ± 1.1), $61.7 \pm 8.9 \times 10^9$ leukocytes/L, $84.7 \pm 64.8 \times 10^6$ CD34/L	0.2 ± 0.1	2	3	SPECT, angiography	↑ LV function, ↑ perfusion, Restenosis: NA	3
STEMMI trial	Double-blind, randomized, placebo-controlled study	33 (26 BMS, 11 DES, 4 no follow-up) /37 (25 BMS, 13 DES, 1 no follow-up)	10 (6), $50.0 \pm 3.0 \times 10^9$ leukocytes/L $53.4 \pm 8.0 \times 10^6$ CD34/L	0.3 (median)	1.2 (median)	6	MRI, echocardiography	No change in LV function, No change in LV size, Restenosis: NS, Elevated circulating VEGFR2 cells and CXCR4 cells by day 7	111

Continued

Table 1 *Continued*

Trial	Study design	Patient (control/test)	G-CSF dose (μg/kg/day)	MI to PCI (days)	PCI to G-CSF (days)	Follow-up (months)	Imaging	Outcomes	Reference
Valgimigli	Single-blind placebo-controlled, randomized study	10/10 (2 patients in each group no PCI, 4 each group had DES)	5 (4), $35 \pm 11 \times 10^9$ leukocytes/L $27.5 \pm 16.7 \times 10^6$ CD34/L	<0.5	1.5 ± 2.7 (symptoms to drug)	6	SPECT	↑ LV function, ↓ LV size, Restenosis:NS	83
Wang	Non-randomized, placebo-controlled study	16/13	5 (6), $49.6 \pm 7.8 \times 10^9$ leukocytes/L 20×10^6 CD34/L	NA	NA	6	SPECT, MRI, echocardiography	↓ LV function, Restenosis: NS	112
Rigenera study	Randomized study	27/14	10 (5), $50.3 \pm 30.2 \times 10^6$ CD34/L	NA	≥5	5	Echocardiography	↑ LV function, ↓ LV size, Restenosis:NA	68
REVIVAL-2	Double-blind, randomized, placebo-controlled study	58 (50 BMS, 8 DES)/ 56 (51 BMS, 5 DES)	10 (5), 48.15×10^9 leukocytes/L $72 \pm 154 \times 10^6$ CD34/L	<12h	5	6	MRI, SPECT, angiography	No change in LVEF, Restenosis: NS	27
Deng	Double-blind, randomized, placebo-controlled study	10/10	10 (7), $6.0 \pm 3.0 \times 10^6$ CD34/L	<12h	NA	12	Echocardiography	↑ LV function ($P<0.05$), No change in LV size, Restenosis: NA	113
Suarez de Lezo	Randomized control groups	–/13	10 (10), $5.5 \pm 1.3 \times 10^{10}$ leukocytes/L $23.0 \pm 22.2 \times 10^6$ CD34/L	0–5 days	5 days after AMI	3	Angiography	↑ LV function, Restenosis: NS	69
Zbinden	Double-blind, randomized, placebo-controlled study	7/7	GM-CSF 10 (14) $31.4 \pm 9.9 \times 10^9$ leukocytes/L	NA	NA	0.5	Flow wire	↑ collateral flow	114
Stem-AMI	Randomized, multi-centre, single-blind open-trial study	8/5	150 (5), $36.1 \pm 2.90 \times 10^9$ leukocytes/L $3 \pm 0.6 \times 10^6$ CD34/L	0.24 ± 12	0.36 ± 0.11	6	Echocardiography, SPECT, MRI, angiography	LV function: NA, Restenosis: NS	115

NA, not applicable; NS, not significant; SPECT, single photon emission computed tomography; MRI, magnetic resonance imaging; GM-CSF, granulocyte macrophage colony stimulating factor.

Table 2 Results of clinical trials using re-infused bone marrow stem cells mobilized by G-CSF

Trial	Patients (control/test)	G-CSF dose (μg/kg/day)	Route, cells	MI to PCI (days)	PCI to G-CSF (days)	Follow-up (months)	Imaging	Outcome	Reference
Boyle	10/5	10 (4)	Intracoronary, $66.9 \pm 17.6 \times 10^6$ $CD34^+$ cells	Old MI	>12months	12	Angiography, SPECT	↑ Symptoms, ↑ Collateral growth, Restenosis: NS	67
MAGIC II	10/10 (G-CSF only) /10 (G-CSF + cell reinfusion) RPC	10 (4)	Intracoronary, $1.5 \pm 0.5 \times 10^9$ leukocytes, $8.3 \pm 10.2\%$ $CD34^+$ cells	Control: 7.1 ± 1.6 (AMI), 117.0 ± 158.6 (OMI) G-CSF: 5.5 ± 3.1 (AMI) 87.3 ± 73.3 (OMI) G-CSF + cell re-infusion: 3.3 ± 1.0 (AMI) 94.6 ± 116.9 (OMI)	Immediately post-PCI	24	SPECT, echocardiography	↑LV function in cell re-infusion group but not G-CSF alone, Restenosis: ↑	116
MAGIC Cell-3-DES	RPC 25/25 (AMI) 16/16 (old MI) all patients with DES	10 (3)	Intracoronary, $1.4 \pm 0.5 \times 10^9$ leukocytes, $9.3 \pm 10.2\%$ $CD34^+$cells	Control: 3.9 ± 4.4 (AMI) 960 ± 832 (OMI) G-CSF + cell infusion: 4.0 ± 3.1 (AMI) 514 ± 524 (OMI)	Immediately post-PCI	6	MRI and angiography	↑LV function in G-CSF+ reinfusion in AMI patients, ↓ LV size in G-CSF+ reinfusion AMI patients, Restenosis: NS	88
Losordo	6/24 RPC	5 (5)	Intramuscular	NA	NA	6	SPECT	↑ Symptoms, ↑ quality of life, Restenosis: NA	117
Steinwender	20	10 (4)	Intracoronary, $4.8 \pm 1.6 \times 10^9$ leukocytes $48.6 \pm 37.2 \times 10^6$ CD34 cells	<0.5	2	6	Angiography, SPECT, echocardiography	↑ LV function, Restenosis: ↑ , 4 patients with DES no restenosis	85
Yaoita	5 iliac crest aspiration, 5 G-CSF apheresis	3–5 (3)	Intramuscular, $3.4 \pm 1.2 \times 10^9$ leukocytes $5.2 \pm 1.6 \times 10^6$ CD34 cells	NA	NA	1	SPECT	No change in LV function, ↑ perfusion, Restenosis:NA	118
GAIN I	6/10	10 (5)	Intracoronary, 50×10^9/L leukocytes $86.54 \pm 12.82 \times 10^6$ CD133 cells	NA	NA	3	SPECT, echocardiography	No change in LV function, Heightened adverse events	119
Ripa	16/32 (16 with VEGF plasmid injection, 16 with VEGF plasmid + G-CSF injection)	10 (6)	Intramuscular (VEGF) $37 \pm 10 \times 10^6$ CD3/L	NA	7 (post VEGF injection)	3	SPECT	No change in LV function, No change in perfusion	120

RPC, randomized placebo-controlled.

Regenerate Vital Myocardium by Activation of Bone Marrow Stem Cells (REVIVAL-2), a randomized, double-blind, placebo-controlled trial, recruited 114 patients with an average age of 60 years. Patients who were diagnosed with ST elevated myocardial infarction (STEMI) and underwent successful reperfusion by PCI were administered G-CSF (10 μg/kg/day) for a period of 5 days.[27] However, REVIVAL-2 failed to support the importance of G-CSF treatment in patients with AMI (*Table 1*). It was observed that patient cohorts between those two trials had substantial differences in age and timing of G-CSF administration post-PCI. The critical difference of patient age could be the most important feature for the differential outcomes reported. Lehrke *et al.* hypothesized that G-CSF/stem cell factor (SCF) therapy may be impaired in older patients.[52] To prove this hypothesis, MI was induced in 6- and 20-month-old rats followed by G-CSF therapy. The G-CSF/SCF therapy could stabilize and reverse the decline in cardiac function, attenuate left ventricular dilation and reduce hypertrophic cardiomyopathies. Interestingly, these changes were not observed in older rats. It was further observed that the degree of reduction in apoptosis was substantially more in young compared with older rats.[52] Increasing evidence also suggests that ageing could impair endogenous cardiac repair mechanisms,[52] reduce angiogenic capacity,[53] diminish doubling abilities,[54,55] and increase cardiomyocyte apoptosis at baseline levels as well as post-ischaemia.[56,57] Furthermore, reports have indicated that there is a significant accumulation of oxidative damage that promotes cell senescence. Generation of free radicals, such as superoxide, hydrogen peroxide, and peroxynitrite, increases with ageing.[58] This is accompanied by decreased activity of anti-oxidant molecules, such as superoxide dismutase and glutathione, that alter the defense mechanism of cells.[59] Ageing is also a well-known limiting factor for the mobilization of BMSCs in donors being treated with G-CSF for leukopheresis for haematopoietic progenitor cells.[60] Therefore, age-related effects are a vital consideration for G-CSF efficacy, and it is important to address this issue in order to benefit elderly patients who, despite reperfusion therapies, have increased MI-related mortality and morbidity.[52]

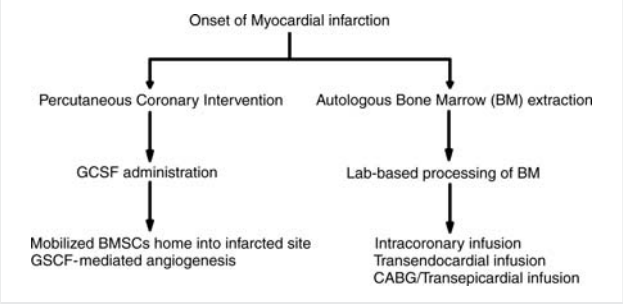

Figure 2 Schematic representation of the methods employed for stem cell therapy in cardiac repair.

2.6 Timing of G-CSF therapy post-MI

Evidence from past clinical trials suggests that besides ageing, the timing of G-CSF administration post-MI could be another critical factor in deciding the outcome of G-CSF therapy. Beneficial effects of G-CSF were significantly better when treatment was initiated by day 3 compared with day 7 post-MI.[7] Indeed, FIRSTLINE-AMI and REVIVAL-2 had a substantial difference in the timing of G-CSF administration after PCI. Contrary to these observations, Overgaard *et al.* reported that the timing of G-CSF administration post-PCI had no significant impact in clinical recovery. G-CSF was administered 17–65 h post-PCI to 27 patients with average age of 58 years (SD 8.8 years), and no statistically significant difference was observed in the left ventricular ejection fraction (LVEF) between the G-CSF and control groups.[61] However, patients enrolled in the study were cases of sub-acute STEMI and late revascularization, thus comparison of these results with other

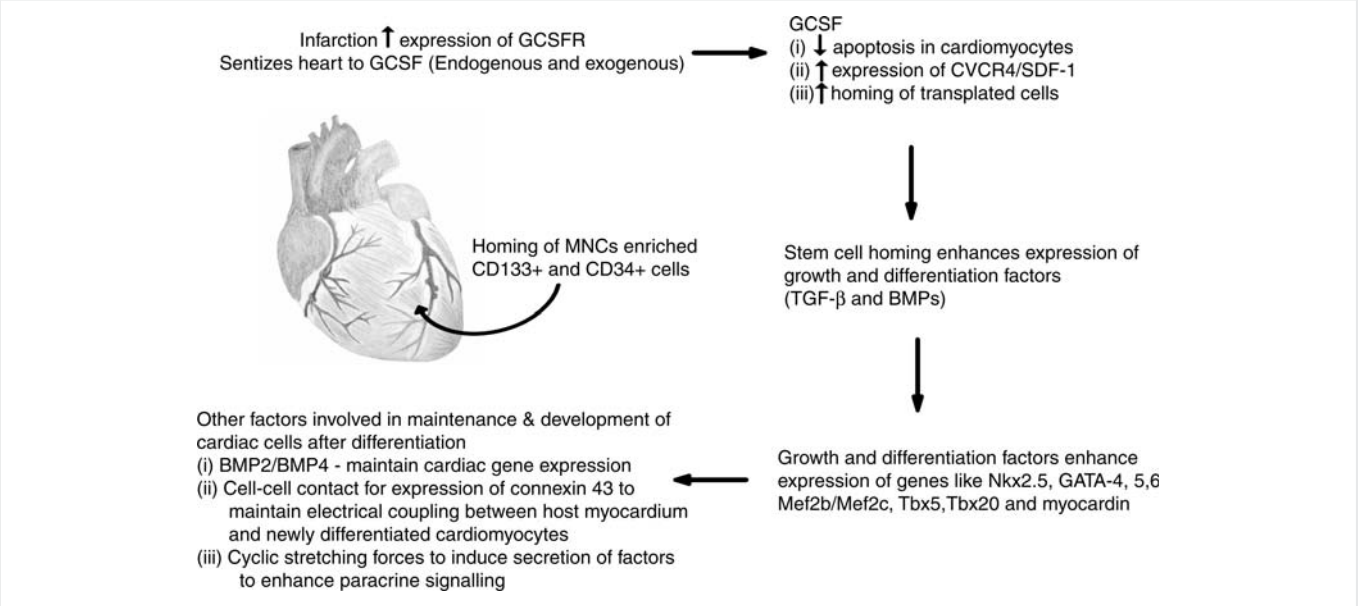

Figure 3 Tentative steps involved in G-CSF-mediated cell therapy.

trials would be difficult. Administration of G-CSF within 24 h of PCI did result in improvement in myocardial perfusion, but not in cardiac function[62] as demonstrated by FIRSTLINE-AMI or animal studies.[63] However, in light of the above findings, it could be suggested that there is a mismatch between activation of homing factors in the injured myocardial tissue, and timing of G-CSF treatment in mobilizing stem cells.[62]

In fact, SDF-1 and other factors, such as VEGF and fibroblast growth factor, increase gradually during the early stages of infarction and reach optimal levels by the third week post-MI.[64] Therefore, early (hours post-MI) administration of G-CSF may not be effective in the recovery of myocardial function[64] due to this temporal mismatch. Furthermore, expression of G-CSFR too is low during the early period of infarction, but gradually increases by day 5 of MI,[65] suggesting that stem cells would be more effective when given or mobilized 5 days after MI.[66] There are a number of clinical studies in which G-CSF administration was delayed after PCI showed signs of improvements in LVEF and infarct size. Boyle *et al.* showed that G-CSF mobilization and administration of $CD34^+$ cells were well tolerated in patients with chronic ischaemic heart disease.[67] They found enhanced formation of collateral vessels, and no in-stent restenosis or proliferative retinopathy after a 12 month collateral follow-up[67] (*Table 2*). The Rigenera study also reported that G-CSF administration 5 days post-PCI was effective in increasing LV function ($P = 0.02$) and reducing infarct size ($P = 0.04$).[68] Similarly, Suarez de Lezo *et al.* reported improved LV function in patients who were administrated G-CSF after 5 days post-PCI.[69] Indeed, a comparative analysis of various clinical outcomes highlighted in *Table 1* and *2* seems to support the benefits of late G-CSF administration.

2.7 G-CSF and route of administration

Low efficacy of G-CSF in clinical trials could also be attributed to the route of administration of stem cells. In most of the clinical trials, biodistribution of BMSCs was not evaluated. Elevated numbers of stem cells have been observed in patients following G-CSF treatment or when cells are injected via the intracoronary route (*Table 2*). The chances of homing of circulating BMSCs in the infarcted region would probably be very low due to the body size ratio and first-pass constraint of the coronary circulation. Recently, in a porcine model, it was demonstrated that BMSCs delivered by the intracoronary route were distributed in the heart and lungs, while intravenously injected BMSCs showed higher lung homing than cardiac engraftment. Furthermore, levels of chemoattractants secreted by the infarcted heart may not favour intravenously injected cells in large species, such as pigs and humans.[70] Similar studies in animals[71] and human patients[72,73] too have shown that only about 1–3% circulating/injected BMSCs homed to the infarcted heart. Furthermore, cell enrichment requires isolation of cells from individual patients that may have considerable variations in the quality (stemness) and composition of cells.

Direct mobilization of stem cells into the peripheral circulation with G-CSF faces similar, if not more, challenges compared with injection of pre-enriched cell populations. These variations may be further affected by associated factors, such as the age of the patients, and other co-morbidities, such as diabetes mellitus, hypercholesterolaemia, hypertension and hyperlipidaemia, which are known to impair the functionality of the stem cells.[74] Moreover, contamination with other cells, such as red blood cells, may further reduce the efficacy of the injected stem cells. *Post hoc* statistical analysis of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial indicated that contamination of the autologous cell products with red blood cells could also negatively impact functional improvement of LVEF in patients.[75] Assmus *et al.* recently confirmed these findings in a porcine model, whereby experimental data demonstrated that red blood cell contamination affected the functionality of BMSCs in a dose-dependent manner.[75] Furthermore, red blood cell contamination altered the mitochondrial potential of stem cells through unknown mechanisms, which affected the stemness of the BMSCs.[75] Therefore, practical and technical issues in relation to clinical operations may exert considerable influence on the eventual outcome of G-CSF efficacy.

2.8 G-CSF and stent restenosis

Contrary to the FIRSTLINE-AMI study, the Myocardial Regeneration and Angiogenesis in Myocardial Infarction with G-CSF and Intracoronary Stem Cell Infusion (MAGIC) study reported a high rate of restenosis in patients who were given G-CSF alone ($n = 3$) or with an adjunctive stem cell infusion ($n = 7$).[76] One of the critical factors suggested for the higher rate of restenosis was treatment with G-CSF for 4 days before reperfusion,[77] which led to raised levels of circulating cells that in turn stimulated an inflammatory reaction and accelerated vascular smooth muscle proliferation before reperfusion and stent implantation.[78,79]

Most patients to whom G-CSF was administered had prior PCI, which may increase the flow of mobilized stem cells towards infarct artery-related territories and possibly enhance re-endothelization.[80,81] Nevertheless, G-CSF may potentially activate neutrophils[82] and possibly contribute to excessive neointimal proliferation and restenosis.[83] Furthermore, an increase in progenitor cells after G-CSF treatment may induce differentiation to smooth muscle cells and contribute to the pathological arterial remodelling, increasing the incidence of restenosis.[76] The type of stent utilized during PCI could also affect the rate of restenosis. Studies have demonstrated that implantation of bare metal stents (BMS) during PCI following transcoronary stem cell transplantation showed higher chances of restenosis[76,84,85] compared with drug-eluting stents (DES), such as sirolimus-eluting stents (SES)[84,86] or paclitaxel-eluting stents (PES).[87] Cho *et al.* using rabbits that underwent iliac artery injury with BMS or PES and received G-CSF for 4 days, showed significantly higher stenosis after 60 days of stenting with BMS.[87] The increased neointimal growth was attributed to proliferation of endothelial and smooth muscle progenitor cells. However, PES preferentially inhibited proliferation of smooth muscle progenitors, thus preventing neointimal hyperplasia.[87] The MAGIC Cell-3-DES trial likewise reported no in-stent restenosis with SES, although there was no comparison with BMS, after G-CSF therapy in 41 patients.[88] A reduction in the incidence of target-vessel failure after 1 year was also noted with SES compared with BMS.[86] Recent reports have suggested that DES interferes with the natural vascular healing by delaying the formation of endothelial lining over the stent.[89,90] Bioengineered GENOUS stents that are coated with CD34 antibody to immobilize circulating endothelial progenitor cells have been developed, and the first human clinical trial with this technology indicates that endothelial progenitor cell-capture stents are safe.[91] A number of recent clinical studies support these findings.[92–94] Interestingly, recent studies have shown that anti-human CD34 immobilized on SES could enhance re-endothelialization compared with SES alone and may potentially be a more effective therapeutic alternative to improve currently available DES.[95] Although to date, there are no reports of this antibody-

coated DES having been used in patients with G-CSF therapy. Meta-analysis studies aimed at evaluating the safety and efficacy of G-CSF have demonstrated that there is no significant risk of restenosis.[96,97] However, LVEF improvement has been inconsistent,[96–99] probably due to different models used for evaluation purposes.

2.9 G-CSF and gender

In almost all clinical trials, about 85 percent of patients recruited have been males. Currently, it would be difficult to predict whether enrolling similar number of patients of both sexes could impact the outcome of these clinical trials.[100] Human and animal studies have shown that the percentage of apoptosis is significantly higher in males than females.[101,102] Furthermore, male myocardium is more prone to the ageing process than female myocardium.[103] Although the exact mechanism for these observations is not clear, estrogen has been postulated to play a role in the outcomes. Estrogen is known to activate the Akt pathway to exert its protective role.[100,104,105] Likewise, G-CSF activates the Akt pathway and may synergize with estrogen in augmenting cardioprotection. This may be harnessed for better clinical outcomes. However, more in-depth studies are needed to evaluate these findings and their implications in future clinical applications.

3. Conclusion

Although the existing regime of G-CSF therapy for AMI has been tried with varying degrees of success, visualization of newer and probably more efficient regimes is warranted. Our group demonstrated, for the first time, that BMSCs could be differentiated efficiently into cardiomyocyte-like cells with defined cardiomyocyte phenotypes.[106] Furthermore, pre-differentiation of stem cells into cardiomyocyte-like cells may potentially enhance their survival and engraftment as cardiomyocytes following myocardial transplantation.[107,108] Utilization of these pre-defined cells along with scaffolds for clinical applications could additionally be benefited by the ability of G-CSF to prime the local milieu and prevent apoptosis of these cells in the grafted area (*Figure 3*).[109]

In conclusion, cardiac repair by G-CSF therapy is a safe therapeutic approach. Based on our understanding, activation of signalling molecules such as SDF-1 may play a crucial role in homing of cells, and delayed administration of G-CSF may be more efficacious. Furthermore, direct injection of cells into the heart by intracoronary infusion may be a better option than intravenous injection because cells infused by latter technique show more lung sequestration. Younger patients may find stem cell therapy more beneficial than older patients because the rate of apoptosis is lower. However, other associated factors, such as the co-morbidities of hyperglycaemia, hypertension, hyperlipidaemia, and confounders, such statin medication, direct and indirect effects of G-CSF, dosage of re-infused cells and infarcted milieu, need to be taken in account to control the variables in future trials to unravel the benefits, if any, of G-CSF stem cell therapy.

Conflict of interest: none declared.

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