Importance of recruitment of bone marrow-derived CXCR4+ cells in post-infarct cardiac repair mediated by G-CSF

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Abstract

Objective: Granulocyte-colony stimulating factor (G-CSF) accelerates repair following myocardial infarction (MI). Recently, the beneficial effects of post-MI administration of G-CSF were reported to be mediated by direct activation of the Jak–Stat pathway in cardiomyocytes. Our aim was to test the hypothesis that bone marrow-derived cells recruited into the infarcted myocardium are the primary mediators of the beneficial effects of G-CSF.

Methods and results: MI was induced using a 30-min ischemia–reperfusion protocol (day 0) in 40 rabbits treated with G-CSF (10 μg/kg/day from days 3 to 7) or saline. Another 40 rabbits received the same G-CSF or saline protocol but also received AMD3100 (200 μg/kg/day), a specific inhibitor of CXCR4. On day 28 post-MI, left ventricular ejection fractions and end-diastolic dimensions were significantly better in the G-CSF group than in the control saline group, and the scar area/left ventricular wall area ratio was significantly smaller in the G-CSF group. G-CSF administration also led to increased mobilization of CXCR4+ bone marrow cells, including RAM11+ macrophages, into infarcted areas. And within those areas there was significant upregulation of expression of stromal cell-derived factor (SDF)-1, a chemoattractant of circulating CXCR4+ cells, as well as of the collagenase matrix metalloproteinase-1. AMD3100 significantly inhibited all of these beneficial effects of G-CSF, but did not affect the upregulation of SDF-1 or phospho-Stat3.

Conclusion: Recruitment of CXCR4+ cells into infarcted myocardial tissues via stimulation of the CXCR4/SDF-1 axis plays a critical role in the beneficial effects of G-CSF.

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Keywords: Myocardial infarction; G-CSF; CXCR4/SDF-1 axis; Post-infarct repair; AMD3100

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1. Introduction

Administration of granulocyte-colony stimulating factor (G-CSF) following myocardial infarction (MI) improves left ventricular (LV) function and reduces LV remodeling and old infarct (scar) size [1–3]. Orlic et al. suggested that cardiomyocyte regeneration was an important mechanism underlying these beneficial effects [1]. On the other hand, we suggested that, given the low incidence of bone marrow (BM)-derived cardiomyocytes, acceleration of the healing process via increased macrophage and cytokine effects (e.g., upregulation of matrix-metalloproteinases (MMPs)) was more likely responsible for the beneficial effect of G-CSF than cardiomyocyte regeneration [2]. In either of these cases, however, the effects of G-CSF are thought to be mediated by BM-derived cells recruited into the infarcted myocardium. A
largely different scenario was recently proposed by Harada et al. [3], who reported that G-CSF exerts its beneficial effects by acting directly on surviving cardiomyocytes and that the effects are mediated via activation of the Jak–Stat pathway.

BM-derived CD34+ cells in the peripheral blood differentiate into cardiomyocytes, smooth muscle cells and vascular endothelial cells after recruitment into infarcted myocardial tissues [4–9]. In addition, circulating CXCR4+ cells, which include CD34+ cells, lymphocytes and monocytes, express a variety of repair-related cytokines, thereby mediating angiogenesis and fibrolysis [4–13]. Among these mediators is stromal cell-derived factor (SDF)-1, a chemoattractant for circulating CXCR4+ cells. Expressed mainly by vascular endothelial and smooth muscle cells within damaged tissues [14], SDF-1 is thought to play a critical role in the mobilization of CXCR4+ cells into cardiac tissues after MI [14–16]. Conversely, AMD3100 is a specific CXCR4 antagonist that disrupts the CXCR4/SDF-1 axis [17,18]. Therefore, to better understand the mechanism by which G-CSF exerts its beneficial effects in post-MI hearts, we used a rabbit ischemia–reperfusion model to test whether AMD3100 would attenuate the beneficial effects of G-CSF by reducing BM cell recruitment into the infarcted myocardium. If G-CSF exerts its effects primarily by acting directly on cardiomyocytes, as suggested by Harada et al. [3], those effects should not be attenuated by inhibition of BM cell recruitment.

2. Materials and methods

All rabbits used in this study received humane care in accordance with the Guide for Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication 8523, revised 1985). The study protocol was approved by the Ethical Committee of Gifu University School of Medicine, Gifu, Japan.

2.1. Ischemia–reperfusion infarct model and post-reperfusion treatment

Male Japanese white rabbits (approximately 2.0 kg) were anesthetized by intravenous administration of 30 mg/kg sodium pentobarbital, after which a 30-min ischemia and reperfusion protocol was carried out as previously described (day 0) [2]. Rabbits surviving 3 days after reperfusion were randomly assigned to one of four groups (n=20 each). Rabbits in the G group were administered G-CSF (10 μg/kg/day) daily for 5 days (from day 3 to day 7 post-MI). Rabbits in the corresponding control group (S group) received an appropriate volume of sterile phosphate buffered saline (PBS) on the same days. Rabbits in the AG and AS groups were respectively administered G-CSF or PBS as above, but also received AMD3100 (200 μg/kg/day, octahydrochloride, Sigma) daily for 7 days (from day 1 to day 7 post-MI) in sterile PBS. The dosage of AMD3100 was determined as described below.

2.2. Blockade of the CXCR4/SDF-1 axis using AMD3100

To determine an appropriate dosage of AMD3100 (octahydrochloride; Sigma) for blockade of the CXCR4/SDF-1 axis, normal rabbits were subcutaneously administered 50, 100, 200 or 300 μg/kg AMD3100 (n=6 in each). Samples of arterial blood was collected before treatment and 3, 6, 12 and 24 h after treatment, and CD34+CXCR4+ cells were counted using an EPICS XL (Beckman Coulter) [17,18]. As shown in the supplementary data, numbers of CD34+CXCR4+ cells reached a dose-dependent peak 12 h after AMD3100 administration, with 200 μg/kg eliciting the greatest effect. At all dosages, the effects of AMD3100 disappeared within 24 h after administration.

2.3. Complete blood cell counts and CD34+CXCR4+ cell counts in peripheral blood

Under anesthesia, arterial blood was collected from the ear arteries of rabbits in the G, S, AG and AS groups (n=10 in each) on days 7 and 28 post-MI. The samples were collected into EDTA-containing tubes supplied by Mitsubishi Biochemical Laboratory (Japan) and were used to obtain complete blood cell counts and hemograms.

For flow cytometric analysis, approximately 2.5 ml of arterial blood were collected using a heparin sodium (500 U)-loaded syringe, after which the mononuclear cell fraction was isolated with mouse anti-human FITC-conjugated CD34 monoclonal antibody (mAb; Serotec Ltd., Oxford, UK) using the protocol provided by the manufacturer (10 μl of mAb solution per 10⁶ cells in 100 μl PBS for 60 min at room temperature) or with mouse anti-human PE-conjugated CXCR4 mAb (R&D Systems, Minneapolis, USA) (10 μl per 10⁵ cells in 100 μl PBS for 60 min at room temperature). The cells were then subjected to flow cytometric analysis using an EPICS XL (Beckman Coulter).

2.4. Echocardiography, heart rate and blood pressure

Prior to infarction and then 28 days post-MI, rabbits in G, S, AG and AS groups (n=10 in each) plus two groups (n=6 in each) of sham-operated rabbits, with and without AMD3100, were anesthetized with sodium pentobarbital (30 mg/kg) and examined using transthoracic echocardiography (Aloka SSD4000) with a 7.5-mHz sector scan probe. Left ventricular (LV) end-diastolic dimension (EDD, mm) and ejection fraction (EF, %) were measured. Heart rate and systemic blood pressure were also measured using a Micro-Tip Catheter transducer™ (SPR320, Millar Instruments, Houston, TX). All of these examinations, as well as the measurements described below, were carried out by two persons (Y.M. and M.A.) blinded to the conditions.
2.5. General pathology

Ten rabbits each in the G, S, AG and AS groups were sacrificed 7 days post-MI using an overdose of pentobarbital after heparinization (500 U/kg), and then the remaining 10 in each group were sacrificed 28 days post-MI. After measuring body weight and LV weight, the left ventricle was sliced into seven transverse sections parallel to the atrioventricular ring from the apex to the base. All seven sections from hearts collected 28 days post-MI and the upper two sections from the center of the infarction in hearts collected 7 days post-MI were fixed in 10% buffered formalin, embedded in paraffin, cut into 4-μm-thick sections, and stained with hematoxylin and eosin (HE) or Sirius red. Using the 28-day preparations, the LV wall areas, infarct areas and scar areas were calculated using an image analyzer connected to a

Fig. 1. Echocardiography illustrating the effect of G-CSF on post-MI cardiac function and remodeling 28 days post-MI. Rabbits receiving G-CSF showed significantly increased LVEF and decreased LVEDD. The improvement in cardiac function and remodeling was inhibited by co-administration of AMD3100. There were no significant differences among the S, G, AS and AG groups with respect to heart rate or systolic and diastolic blood pressures before or 28 days post-MI.
light microscope (LUZEX-F, NIRECO, Tokyo) and expressed as mm$^2$/slice/body weight (kg).

2.6. Immunohistochemistry

Sections from the same paraffin-embedded preparations described above were also used for immunohistochemical analyses. Using the indirect immunoperoxidase method, immunohistochemical staining was carried out with mouse anti-human CXCR4 mAb (1:250, R&D Systems), mouse anti-rabbit macrophage mAb (1:100, DAKO-RAM 11), mouse anti-human $\alpha$-smooth muscle actin mAb (1:250, DAKO-smooth muscle actin, 1A4), mouse anti-human endothelial cell CD31 mAb (1:100, DAKO) and mouse anti-human/mouse SDF-1 mAb (1:250, R&D Systems), each of which cross-reacts with rabbit tissues. Macrophages are one type of CXCR4$^+$ cell [19].

2.7. Western blot analysis

Immediately after sacrifice 7 days post-MI, the infarcted LV region including the border zone, and a remote non-infarcted region, were individually snap-frozen for Western blot analysis. Fifty milligrams of frozen tissues from each heart were homogenized in lysis buffer and centrifuged for 10 min at 10,000×g and 4 °C. Levels of SDF-1, vascular endothelial growth factor (VEGF) and MMP-1 were then assessed by Western blot analysis of tissue specimens from the infarcted area of the LV wall using mouse anti-human SDF-1 mAb (1:250, R&D Systems), mouse anti-human VEGF mAb (1:250, Research Diagnostics Inc.) and mouse anti-human MMP-1 mAb (1:500, Daiichi Fine Chemical Co., Ltd., F-67), respectively. Following MI, these molecules are thought to be expressed mainly in the infarcted tissues, where they play important roles in tissue repair [2]. We also used a polyclonal rabbit anti-mouse phospho-Stat3 Ab (1:100, Cell Signaling) to assess expression of phospho-Stat 3, which is produced mainly by cardiomyocytes in both infarcted and non-infarcted areas [3]. Levels of phospho-Stat3 were measured in non-infarcted LV regions so as to exclude the effects of G-CSF-induced reduction in infarct size and its inhibition by AMD3100 on Stat3 expression. Signals were quantified by densitometry.

Fig. 2. Peripheral blood cell counts. Rabbits administered G-CSF showed higher WBC (A), mononuclear cell (B) and CD34$^+$CXCR4$^+$ cell (C) counts 7 days post-MI. AMD3100 had no effect on cell counts. (D) Serum SDF-1α levels were higher in rabbits administered G-CSF than in those administered PBS, although or not AMD3100 was co-administered.
2.8. Serum SDF-1α

Using blood samples collected as described in Section 2.3, serum was obtained from the uppermost layer of samples after centrifugation on a ficoll gradient. Serum SDF-1α levels were measured using a commercially available ELISA kit (R&D Systems) in cooperation with Mitsubishi Biochemical Laboratory (Tokyo, Japan).

2.9. Statistical analysis

All values are presented as the means±S.D. Differences between groups were assessed using two-way repeated measures of analysis of variance (ANOVA) with a post hoc Tukey–Kramer's test. Values of \( p < 0.05 \) were considered significant.

3. Results

3.1. Mortality

Six rabbits died within 24 h after MI was induced using a 30-min ischemia and reperfusion protocol. However, all rabbits assigned to each group 3 days after reperfusion survived for as long as required, 7 or 28 days.

3.2. Effect of G-CSF and AMD3100 on cardiac function and hemodynamics following induction of MI

Echocardiographic examination showed that on day 28 post-MI, LVEF and LVEDD were significantly better in rabbits treated with G-CSF (G group: \( 62.6\pm6\% \) and \( 10.5\pm0.9 \) mm, respectively) than in those treated with PBS (S group: \( 50.5\pm5\% \) and \( 13.6\pm1.0 \) mm, respectively) (Fig. 1). The beneficial effects of G-CSF on LVEF and LVEDD were completely blocked by co-administration of AMD3100, a specific inhibitor of CXCR4, along with G-CSF. This inhibitory effect of AMD3100 was specific for G-CSF, as there was little or no difference in LVEF and LVEDD in the S and AS groups. There also were no significant differences among the S, G, AS and AG groups with respect to heart rate or systolic and diastolic blood pressures before or 28 days post-MI (Fig. 1). And AMD3100 had no effect on cardiac function or hemodynamics in sham-operated rabbits (\( n=6 \), data not shown).

Fig. 3. LV weight, LV wall area, scar area and transverse cardiomyocyte diameters 28 days post-MI. G-CSF reduced scar areas measured in sections stained with Sirius red and this effect was inhibited by AMD3100. There were no significant differences in LV weight, LV wall areas or cardiomyocyte transverse diameters among the G, S, AG and AS groups. The border area was defined as surviving myocardial tissue within 1 mm of the infarcted area.
3.3. Effects of G-CSF and AMD3100 on WBC, mononuclear cell and CD34+CXCR4+ cell counts and SDF-1 levels in circulating blood

The numbers of WBCs, mononuclear cells and CD34+CXCR4+ cells in circulating blood were significantly higher on day 7 post-MI in the G group than in the S group, though these differences had disappeared by day 28 post-MI (Fig. 2). Serum SDF-1 also was significantly higher in the G group than the S group. Co-administration of AMD3100 with G-CSF or PBS (AG and AS groups, respectively) had no effect on WBC, mononuclear cell or CD34+CXCR4+ cell counts, or on serum SDF-1 levels (Fig. 2).

3.4. Effects of G-CSF and AMD3100 on the general histopathology in the heart following MI

Seven days post-MI, the infarcted areas of the LV wall contained granulation tissue comprised of myofibroblasts and small vessels; scar tissue comprised of collagen and fatty cells was seen 28 days post-MI. On day 28 post-MI, the LV weights and LV wall areas were similar in the S and G groups; however, the Sirius red+ scar-to-LV wall area ratios were significantly smaller in the G group (9.1 ± 2.3%) than in the S group (18.3 ± 2.6%) (Fig. 3). The reduction in scar size seen in the G group was not seen in the AG group, though the scar sizes were similar in the S and AS groups. In addition, the numbers of CD31+ capillaries and α-smooth muscle actin+ myofibroblasts were significantly higher in the infarcted areas in the G group than in the S group on day 7 post-MI, and these increases were blocked in the AG group (Fig. 4). Finally, the transverse diameters of the cardiomyocytes were significantly larger in areas bordering the infarction than in remote non-infarcted areas, and there was no significant difference between the S and G groups (Fig. 3). In non-infarcted areas, there were no significant differences in collagen content or transverse diameters of cardiomyocytes among the S, G, AG and AS groups (Fig. 3).
3.5. Effects of G-CSF and AMD3100 on myocardial CXCR4+ cell counts and SDF-1 within infarcted areas following induction of MI

As shown in Fig. 4, the numbers of RAM 11+ macrophages and CXCR4+ cells within the infarcted areas 7 days post-MI were significantly greater in the G group (28 ± 6 and 54 ± 4 cells/400× HPF, respectively) than in the S group (18 ± 3 and 40 ± 5 cells/400× HPF, respectively). Again, the inhibitory effect of AMD3100 was selective for G-CSF activity, as it blocked the G-CSF-induced increases in cell numbers in the AG group (14 ± 4 and 25 ± 6 cells/400× HPF),

**Fig. 4 (continued).**

3.5. Effects of G-CSF and AMD3100 on myocardial CXCR4+ cell counts and SDF-1 within infarcted areas following induction of MI

As shown in Fig. 4, the numbers of RAM 11+ macrophages and CXCR4+ cells within the infarcted areas 7 days post-MI were significantly greater in the G group (28 ± 6 and 54 ± 4 cells/400× HPF, respectively) than in the S group (18 ± 3 and 40 ± 5 cells/400× HPF, respectively). Again, the inhibitory effect of AMD3100 was selective for G-CSF activity, as it blocked the G-CSF-induced increases in cell numbers in the AG group (14 ± 4 and 25 ± 6 cells/400× HPF),

**Fig. 5.** Immunohistochemical detection of SDF-1 within the infarcted myocardium 7 days post-MI. Note the upregulation of SDF-1 expression (brown in B) in the G group, as compared with the S group (brown in A). The upregulation of SDF-1 was not affected by AMD3100 (brown in C). SDF-1 expression (brown in D1 and E1) is observed in CD31+ vascular endothelial cells (brown in D2) and α-smooth muscle actin+ vascular smooth muscle cells (brown in E2).
Fig. 6. Western blot analysis 7 days post-MI. The level of SDF-1 expression (A), which was upregulated in infarcted areas on day 3 post-MI, remained higher in the G group than the S group 7 days post-MI and was not affected by AMD3100 (AG group). G-CSF also induced upregulation of VEGF (B) and MMP-1 (C) in infarcted areas, but those effects were inhibited by AMD3100. G-CSF also induced upregulation of phospho-Stat3 (D) in non-infarcted LV regions, and that effect, too, was not inhibited by AMD3100.
respectively), but cell numbers were similar in the S and AS groups.

Administration of G-CSF led to upregulation of SDF-1 expression in vascular endothelial and smooth muscle cells within infarcted areas, which was not affected by co-administration of AMD3100 (G group, 9.8±1.3; S group, 4.6±0.4; and AG group, 8.8±1.6) (Figs. 5 and 6). As with the other factors measured, the levels of SDF-1 expression were similar in the S and AS groups (3.6±1.0).

3.6. Effects of G-CSF and AMD3100 on myocardial expression of VEGF, MMP-1 and phospho-Stat3

Administration of G-CSF also led to upregulation of VEGF and MMP-1 expression within infarcted areas, but in contrast to SDF-1 co-administration of AMD3100 completely blocked the G-CSF-induced upregulation of both VEGF (G group, 2.8±0.7; S group, 1.2±0.4; AG group, 1.1±0.7) and MMP-1 (G group, 18.1±2.4; S group, 8.7±3.8; AG group, 4.5±0.2) expression on day 7 post-MI (Fig. 6).

Also upregulated by G-CSF was phospho-Stat3 in non-infarcted areas and that effect was not inhibited by AMD3100 (G group, 9.5±2.0; AG group, 8.6±2.5).

4. Discussion

4.1. Mobilization of CXCR4+ cells into infarcted tissues and activation of the CXCR4/SDF-1 chemotactic axis by G-CSF

We have shown that G-CSF enhances mobilization of CXCR4+ cells from bone marrow into circulation and recruitment of circulating CXCR4+ cells from circulation into the infarcted myocardium, and that AMD3100, an antagonist of CXCR4 which can block the binding between CXCR4+ cells and SDF-1, a chemoattractant for circulating CXCR4+ cells [20], inhibited the recruitment of CXCR4+ cells from the circulating blood into the infarct tissues. Taken together, our findings strongly suggest that the CXCR4/SDF-1 axis plays an important role in the G-CSF-mediated recruitment of CXCR4+ cells into the infarcted myocardium.

According to an earlier paper [14], SDF-1 expression in infarcted tissue of saline-treated animals was enhanced on day 3 post-MI, as compared to a sham group, but the upregulation disappeared by day 7, which was confirmed by the present data shown in the saline group. In the present study, the administration of G-CSF upregulated the expression level of SDF-1 in infarcted tissues by 7 days post-MI, compared with saline injection. In addition, the SDF-1 expression was observed in the endothelial or smooth muscle cells in infarct areas. However, while AMD3100 inhibited the recruitment of CXCR4+ cells from the circulating blood into the infarct tissues, AMD3100 did not affect the upregulation of SDF-1 in the infarct tissues, indicating that the upregulation of SDF-1 by G-CSF is independent of the recruited bone marrow cells in infarct tissues. This supports the claim of Askari et al. that SDF-1 is important for the recruitment of G-CSF-mobilized BM stem cell into infarcted tissues [21].

PET et al. reported that the effects of a SDF-1 neutralizing Ab and AMD3100 differ in some ways, though both block the CXCR4/SDF-1 axis [22]. Briefly, whereas the SDF-1 neutralizing Ab efficiently reduces stem and progenitor cell mobilization, AMD3100 transiently increases cell mobilization from bone marrow into peripheral blood, though the cell counts in peripheral blood return to control levels within 24 h (see the supplementary data). This would explain no effect of AMD3100 on the circulating CD34+CXCR4+ cells in the saline or G-CSF group, which was assessed 24 h after AMD3100 administration.

4.2. Mobilization of CXCR4+ cells into infarcted tissues and the beneficial effects by G-CSF

It is well established that G-CSF administration improves cardiac function and remodeling after myocardial infarction in animal models [1–3]. It also enhances mobilization of BM cells into the peripheral circulation and then from the circulation into infarcted tissues, reduces old infarct size, and induces expression of a variety of cytokines and enzymes (e.g., MMPs) [1,2]. In the present rabbit infarct model, the CXCR4 antagonist AMD3100 inhibited all of the beneficial effects of G-CSF except mobilization of CXCR4+ cells from BM into the circulation. Moreover, it also did not affect the activation of the Jak–Stat pathway in cardiomyocytes, which were confirmed to be CXCR4+ and SDF-1+ by ourselves and others [14,23]. That AMD3100 can interfere with CXCR4+ cells but not with cardiomyocytes further supports the idea that the mechanism underlying the beneficial effects of G-CSF involves enhanced recruitment of CXCR4+ cells into infarcted tissues.

On the other hand, Harada et al. [3] recently claimed that G-CSF receptors are present on cardiomyocytes, that G-CSF activates the Jak–Stat pathway, and that the improvement in cardiac function and reduction in remodeling induced by G-CSF is not seen in a MI model using mice whose cardiomyocytes express a dominant-negative Stat 3 mutant, though mobilization of BM cells is unaffected. Based on those findings, they proposed that the beneficial effects of G-CSF are exerted directly on cardiomyocytes via activation of Stat 3. In the present study, however, AMD3100 inhibited the beneficial effects of G-CSF, despite exerting no effect on the increased activation of Stat3 seen in the G-CSF group but not the saline group.

The discrepancy between the findings of Harada et al. [3] and ourselves may mean that neither the direct effects of G-CSF on cardiomyocytes nor its ability to mobilize BM cells is sufficient to elicit all the beneficial post-MI effects of G-CSF. Instead, the observed therapeutic efficacy...
of G-CSF may reflect both of those modes of action. A second possibility is that the dominant-negative Stat3 mutant used by those investigators to inhibit the Jak–Stat pathway in cardiomyocytes also inhibited G-CSF-induced SDF-1 expression in vascular endothelial and smooth muscle cells. In addition, we recently reported that there are Jak–Stat+ cells present among the interstitial cells within scar areas [24]. If so, an AMD3100-sensitive cytokine effect mediated by Jak–Stat+ and CXCR4+ interstitial cells may also contribute to the beneficial effects of G-CSF. A third possibility is that AMD3100 also inhibits downstream mediators of Stat3 in the Jak–Stat pathway, although AMD3100 is generally thought to be a specific CXCR4 antagonist. Finally, the role of the Jak/Stat pathway may differ in the ischemia (permanent occlusion) model used by Harada et al. [3] and the present study. Mascareno et al. reported that inhibition of Jak2 improves cardiac function after ischemia and reperfusion [25]. Thus, the discrepancy between Harada et al. and ourselves could be explained if activation of the Jak-Stat pathway is independent of the G-CSF-induced beneficial effects in ischemia–reperfusion model.

4.3. Mobilization of CXCR4+ cells and VEGF, CD31+ capillaries or α-smooth muscle actin+ myofibroblasts

CXCR4+ cells, including CD34+ cells, are known to be associated with angiogenesis and myogenesis, acting via paracrine effects as well as cell differentiation [4–8]. Moreover, G-CSF reportedly induces expression of the angiogenic cytokine VEGF and increases numbers of CD31+ capillaries and α-smooth muscle actin+ myofibroblasts [2,26], all of which were confirmed by our findings obtained 7 days post-MI. The attenuation of G-CSF-induced angiogenesis and myogenesis by AMD3100 suggests that CXCR4+ cells recruited into the infarcted myocardium modulate VEGF expression, angiogenesis and myofibroblast proliferation.

4.4. Mechanism by which AMD3100 inhibits G-CSF-mediated reduction in scar size

We found that G-CSF stimulated expression of MMP-1 collagenase and reduced infarct scar size, which is consistent with our earlier results [2]. AMD3100 inhibited both of these effects and also reduced the numbers of collagen-producing myofibroblasts within the infarcted tissues, but there was no correlation between myofibroblast numbers and the scar area on day 28 post-MI (p=0.375) (data not shown). Most likely, therefore, the inhibition of G-CSF-induced reduction in scar size by AMD3100 reflects a decrease in MMP-1 expression rather than an acceleration of collagen production by myofibroblasts. Conversely, this finding strongly suggests that upregulation of MMP-1 underlies the G-CSF-mediated reduction in scar size, which is consistent with our hypothesis that excessive fibrosis and the resultant reduction in cardiac function would accelerate cardiac remodeling in the infarcted heart [2]. An increase in MMP family collagenases may exert a protective effect via degradation of excess collagen.

4.5. Clinical implication

Improvements in cardiac function mediated by G-CSF have been seen using both of the ischemia–reperfusion and permanent occlusion models in animal experiments [2,3]. In studies on human subjects, Ince et al. [27] and we [28] found that subcutaneous injection of G-CSF after reperfusion of the infarct-related coronary artery significantly improves post-infarct cardiac function and is safe, though there was no placebo group in these studies. On the other hand, Ripa et al. recently carried out the first double-blind, placebo-controlled trial of G-CSF and reported that G-CSF treatment after reperfusion following acute myocardial infarction is safe, but did not lead to further improvement in ventricular function, as compared to the recovery observed in the placebo group [29]. The discrepancy between these clinical trials may be explained by a placebo effect and/or differences in clinical factors such as the dose of G-CSF used and the time point at which G-CSF was administered, among others. All that we can say is that the improvement in cardiac function and remodeling induced by G-CSF was not dramatic in any of the human infarct trials, irrespective of whether a significant difference from control was detected. Indeed, the G-CSF effect also was not dramatic in animal studies [2,3] including the present study, although a significant difference from control was seen. We therefore suggest that development of new methods, such as combination therapies using G-CSF and other agents, should be investigated.

5. Conclusion

CXCR4+ cells recruited into infarcted myocardial tissues via stimulation of the CXCR4/SDF-1 chemotactic axis are key mediators of the improvement in cardiac function and the reduction in scar tissue seen after treatment with G-CSF.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cardiores.2006.05.002.

References


