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Modification of Post-Myocardial Infarction Granulocyte-Colony Stimulating Factor Therapy With Myelosuppressives

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Background The purpose of the present study was to investigate the effect of granulocyte colony-stimulating factor (G-CSF) in combination with myelosuppressives on post-myocardial infarction (MI) myocardial repair.
Methods and Results Twenty-four hours after 30-min ischemia and reperfusion (day 0), rabbits were assigned to 4 treatment groups: myelosuppressives (M group), G-CSF (G group), the 2 in combination (MG group) or saline (S group). Significantly greater numbers of circulating stem cells were seen in the MG group than in the G group, with attenuated leukocytosis. In addition, MG caused the greatest upregulation of stromal cell-derived factor (SDF)-1 within the infarcted myocardium and thus recruitment of stem cells from the circulation into the infarcted tissue. This led to enhanced myocardial repair, as indicated by the numbers of bone marrow cell-derived cardiomyocytes and endothelial cells, reduction in scar tissue, improvement in cardiac function and reduction in left ventricular remodeling during the chronic phase of MI. These beneficial effects were entirely abolished by the administration of a CXCR4 antagonist AMD3100, which indicates the importance of CXCR4/SDF-1-axis as a mechanism underlying myocardial repair.
Conclusion The combination of G-CSF and myelosuppressives may be a useful new therapy that overcomes the insufficiency seen with G-CSF alone. (*Circ J* 2007; **71:** 580–590)

Key Words: Cardiac repair; CXCR4/SDF-1 axis; G-CSF; Myelosuppressives; Myocardial infarction

The administration of granulocyte-colony stimulating factor (G-CSF) following myocardial infarction (MI) reportedly diminishes left ventricular (LV) remodeling and improves cardiac function via mechanisms that include myocardial regeneration and acceleration of the healing process, among others.[1–4] Underlying those beneficial effects is, mainly, the capacity of G-CSF to induce recruitment of bone marrow-derived stem/progenitor cells (BMSCs) to the infarcted myocardium. Nevertheless, recent clinical trials to investigate the post-MI administration of G-CSF have shown that the therapy led to no improvement in cardiac function and LV remodeling, although treatment with G-CSF is safe.[5] Accordingly, we have been investigating alternative ways to use G-CSF, including in combination with other agents.

It is general knowledge in the fields of hematology and oncology that myelosuppressives such as 5-fluorouracil and cyclophosphamide mobilize BMSCs, and that this effect is augmented by adding G-CSF.[6,7] We previously reported that post-MI treatment with myelosuppressives induces $CD34^+$ cell mobilization via mild myelosuppression, reduces scar tissue and protects against LV remodeling.[8] We therefore hypothesized that the administration of myelosuppressives prior to G-CSF amplifies the effects of G-CSF alone on BMSC recruitment and post-MI myocardial repair.

Stromal cell-derived factor (SDF)-1 expressed within the infarcted myocardium is well known to play a critical role in attracting mobilized CXCR4-positive BMSCs.[2,9] $CXCR4^+$ cells contain not only $CD34^+$ cells but also inflammatory cells such as monocytes/macrophages,[10,11] and the cells mobilized via upregulation of the CXCR4/SDF-1 axis and recruited into infarcted myocardial tissue stimulate the post-MI healing process via paracrine effects and/or myocardial regeneration.[1,2] Bearing that in mind, we investigated the role played by the activated CXCR4/SDF-1 axis in the beneficial effects of post-MI administration of myelosuppressives and G-CSF using the specific CXCR4 antagonist AMD3100, which disrupts recruitment of $CXCR4^+$ cells, including stem cells and macrophages, into targeted tissues.[12]

Methods

Rabbits used in the present study received humane care in accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 83-23, revised 1996). The study protocol was approved by the Ethical Committee of Gifu University School of Medicine, Japan.

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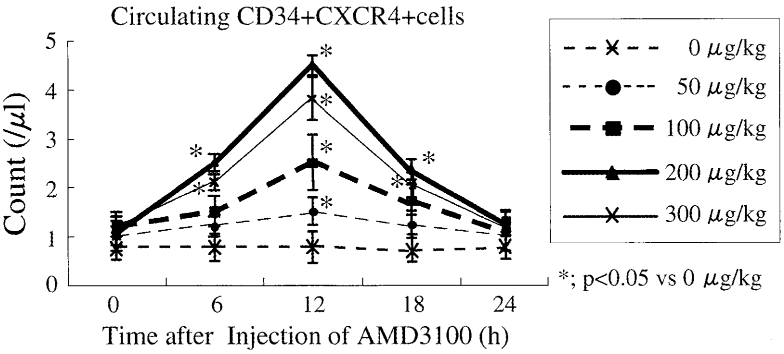

Fig 1. Characteristics of AMD3100. To determine an appropriate dosage of AMD3100 (a specific antagonist of CXCR4; octahydrochloride, Sigma) to block the CXCR4/stromal cell-derived factor-1 axis, normal rabbits were subcutaneously treated with 0, 50, 100, 200 or 300 μg/kg of AMD3100 (n=6 in each). The $CD34^+CXCR4^+$ cells dose-dependently peaked at 12 h after the injection in rabbits receiving 50, 100 or 200 μg/kg, and dropped on the baseline at 24 h after injection. However, 300 μg/kg worked less effectively than 200 μg/kg.

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).[1] Briefly, under anesthesia and mechanical ventilation with room air, a left thoracotomy was performed, and 4-0 silk string was placed beneath the large coronary arterial branch coursing down the middle of the anterolateral surface of the LV. The 30-min coronary arterial occlusion and reperfusion was then performed by tightening and then releasing a snare made with the silk string. Rabbits surviving 24 h after reperfusion were randomly assigned to one of 8 groups (n=20 in each). Rabbits in the MG group were intravenously injected with 5-fluorouracil ($5\,mg \cdot kg^{-1} \cdot day^{-1}$) and cyclophosphamide ($20\,mg \cdot kg^{-1} \cdot day^{-1}$) on days 1 and 2; G-CSF (G; $10\,\mu g \cdot kg^{-1} \cdot day^{-1}$) was subcutaneously injected on the following 5 days (days 3 to 7). Rabbits in the M and G groups received either 5-fluorouracil/cyclophosphamide or G-CSF, respectively. Rabbits in the corresponding control (S) group received an appropriate volume of saline on the same days.

Co-Administration of AMD3100 for Blockade of the CXCR4/SDF-1 Axis

To determine an appropriate dosage of the specific CXCR4 antagonist AMD3100 (octahydrochloride, Sigma) with which to block the CXCR4/SDF-1 axis, normal rabbits were subcutaneously treated with 0, 50, 100, 200 or 300 μg/kg AMD3100 (n=6 in each). Their arterial blood was collected before treatment and 3, 6, 12, 18 or 24 h after treatment, and the $CD34^+CXCR4^+$ cells were counted using an EPICS XL (Beckman Coulter). As shown in Fig 1, the numbers of $CD34^+CXCR4^+$ cells peaked dose-dependently 12 h after injection in rabbits receiving 0, 50, 100 or 200 μg/kg AMD3100 and then returned to baseline within 24 h. Because AMD3100 was less effective at a dosage of 300 μg/kg than at 200 μg/kg, the latter was used to block the CXCR4/SDF-1 axis. Based on the data obtained, the remaining 80 rabbits, which were otherwise treated in the same manner as those in the MG, M, G and S groups, respectively, also simultaneously received the determined dose of AMD3100 ($200\,\mu g \cdot kg^{-1} \cdot day^{-1}$) in saline daily from day 1 to 7 (AMG, AM, AG and AS groups; n=20, in each).

Complete Blood Cell Counts (CBC) and $CD34^+CXCR4^+$ Cell Counts in Peripheral Blood

Under anesthesia, arterial blood was collected from the ear arteries of rabbits in the MG, M, G and S groups (n=10, in each) on days 0, 3, 7, 14, 21 and 28 post-MI, and CBC and hemograms were obtained in co-operation with Mitsubishi Biochemical Laboratory (Japan).

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 on a ficoll gradient (JIMRO, Takasaki, Japan). After hemolysis of contaminating erythrocytes using ammonium chloride (Stem Cell Technologies), approximately 1.5×10^5 mononuclear cells were incubated with mouse anti-human FITC-conjugated CD34 monoclonal antibodies (mAb; Serotec) using the protocol provided by the manufacturer (10 μl of mAb solution per 10^6 cells in 100 μl phosphate-buffered saline (PBS) for 60 min at room temperature) or with mouse anti-human PE-conjugated CXCR4 mAb (R&D Systems) (10 μl per 10^5 cells in 100 μl PBS for 60 min at room temperature).[2]

Echocardiography

Prior to infarction and then 28 days post-MI, 10 rabbits in each group were placed under sodium pentobarbital (30 mg/kg) anesthesia and examined using transthoracic echocardiography (M-mode, Aloka SSD4000) with a 7.5-mHz sector scan probe. The LV end-diastolic dimension (EDD, mm) and ejection fraction (EF, %) were measured.

General Pathology

Ten rabbits in each group were killed with an overdose of pentobarbital after heparinization (500 U/kg) 7 days post-MI, and the remaining 10 in each group were killed 28 days post-MI. After measuring the body weight and LV weight, the LV was sliced into 7 transverse sections parallel to the atrioventricular ring from the apex to the base. All 7 sections from the hearts collected 28 days post-MI and the upper 2 sections from the center of the infarction in the hearts collected 7 days post-MI were fixed in 10% buffered formalin, embedded in paraffin, sliced into 4-μm-thick sections, and then stained with hematoxylin and eosin or Sirius red. Using 7 preparations collected 28 days post-MI, LV wall areas, infarct areas, surviving areas, and scar areas were calculated using a light microscope-connected image analyzer (LUZEX-F, NIRECO, Tokyo). Values are expressed as mm^2/slice and are mean values of 7 slices per heart.

Immunohistochemistry

Sections from the paraffin-embedded preparations described above were also used for immunohistochemistry. Using the indirect immunoperoxidase method, immunohistochemistry 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 α-smooth muscle actin mAb (1:250, DAKO), mouse

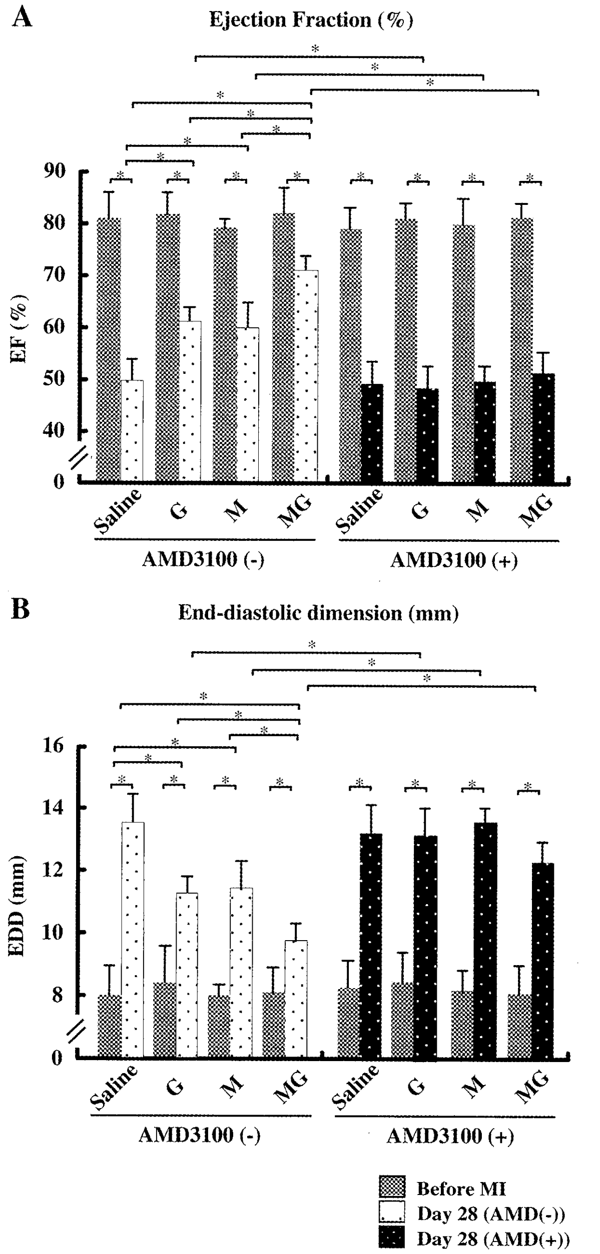

Fig 2. Echocardiography illustrating the effect of granulocyte-colony stimulating factor (G-CSF) and/or myelosuppressives on cardiac function and remodeling 28 days post-myocardial infarction (MI). Rabbits receiving G-CSF and/or myelosuppressives showed a significantly increased left ventricular ejection fraction (EF) and a decreased left ventricular end-diastolic dimension (EDD), than the saline group. Note that the improvement in the cardiac function and remodeling was the greatest in the myelosuppressive-combined G-CSF group and the effects were inhibited by AMD3100.

anti-human endothelial cell CD31 mAb (1:100, DAKO) and mouse anti-human/mouse SDF-1 mAb (1:250, R&D Systems).[1,2]

In Situ Detection of Nuclear DNA Fragmentation (TUNEL)

DNA fragments were labeled in deparaffinized 4-μm-thick sections from a transverse tissue block using an ApopTag in situ apoptosis detection kit (Chemicon-S7100), according to the manufacturer's instructions.[13]

Western Blotting

Immediately after collecting and sectioning the hearts from rabbits killed 7 days post-MI, infarcted areas of the lowest 2 sections were snap-frozen for Western blotting. Fifty milligrams of frozen tissue 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 matrix-metalloproteinase (MMP)-1 were then assessed by Western blotting using mouse anti-human SDF-1 mAb (1:250, R&D Systems), mouse anti-human VEGF mAb (1:250, Research Diagnostics), mouse anti-human MMP-1 mAb (1:500, Daiichi Fine Chemical), respectively, after which the signals were quantified by densitometry.

DiI-Labeling of Bone Marrow (BM)-Derived Mononuclear Cells and Laser Scanning Confocal Microscopy

In 48 rabbits under anesthesia, 10 ml of iliac BM were aspirated and suspended in PBS containing 2,000 U of heparin sodium. BM-derived mononuclear cells (BM-MNCs) were then isolated by centrifugation on a ficoll gradient, and approximately 1.0×10^8 BM-MNCs were suspended in 5 ml of PBS. To label the cells 1,1'-dioctadecyl-1 to 3,3,3',3'-tetramethylindocarbocyanine-perchlorate dye (DiI, Molecular Probes) was added to the BM-MNCs, and then reinjected into the iliac bone! Thereafter, 6 rabbits in each group were subjected to the 30-min ischemia and reperfusion protocol. Twenty-eight days post-MI, the rabbits were killed and their hearts were immediately excised and placed in iced PBS. For immunohistocmemical analysis by confocal microscopy, samples (approximately 3×3×2 mm) obtained from the center of the infarcted anterior LV wall were embedded in OCT compound (Miles Scientific), snap-frozen in liquid nitrogen and sliced into 6-μm-thick sections using a cryostat. Immunofluorescence microscopy (LSM510 NLO, Zeiss) was carried out using mouse anti-troponin I mAb (1:5,000, Chemicon), mouse anti-human endothelial cell CD31 mAb (1:100, DAKO) and Hoechst 33342 for nuclear staining.

Statistical Analysis

All values are presented as the means ± SD. Differences between groups were assessed using two-way repeated measures of analysis of variance with a post-hoc Tukey-Kramer's test. Values of $p<0.05$ were considered to be significant.

Results

Mortality

All rabbits assigned to the MG, M, G, S, AMG, AM, AG or AS group 24 h after reperfusion survived the required 7 or 28 days.

Echocardiography

On day 28 post-MI, LVEF and LVEDD were significantly better in the MG group than in the G, M or S group, although the G and M groups showed significant improvement, as compared to the S group (Fig 2). These improvements were absent in the AMG, AM and AG groups, which also received AMD3100.

CBC, Hemograms and $CD34^+CXCR4^+$ Cells in Peripheral Blood

As shown in Fig 3, the leukocyte and granulocyte counts

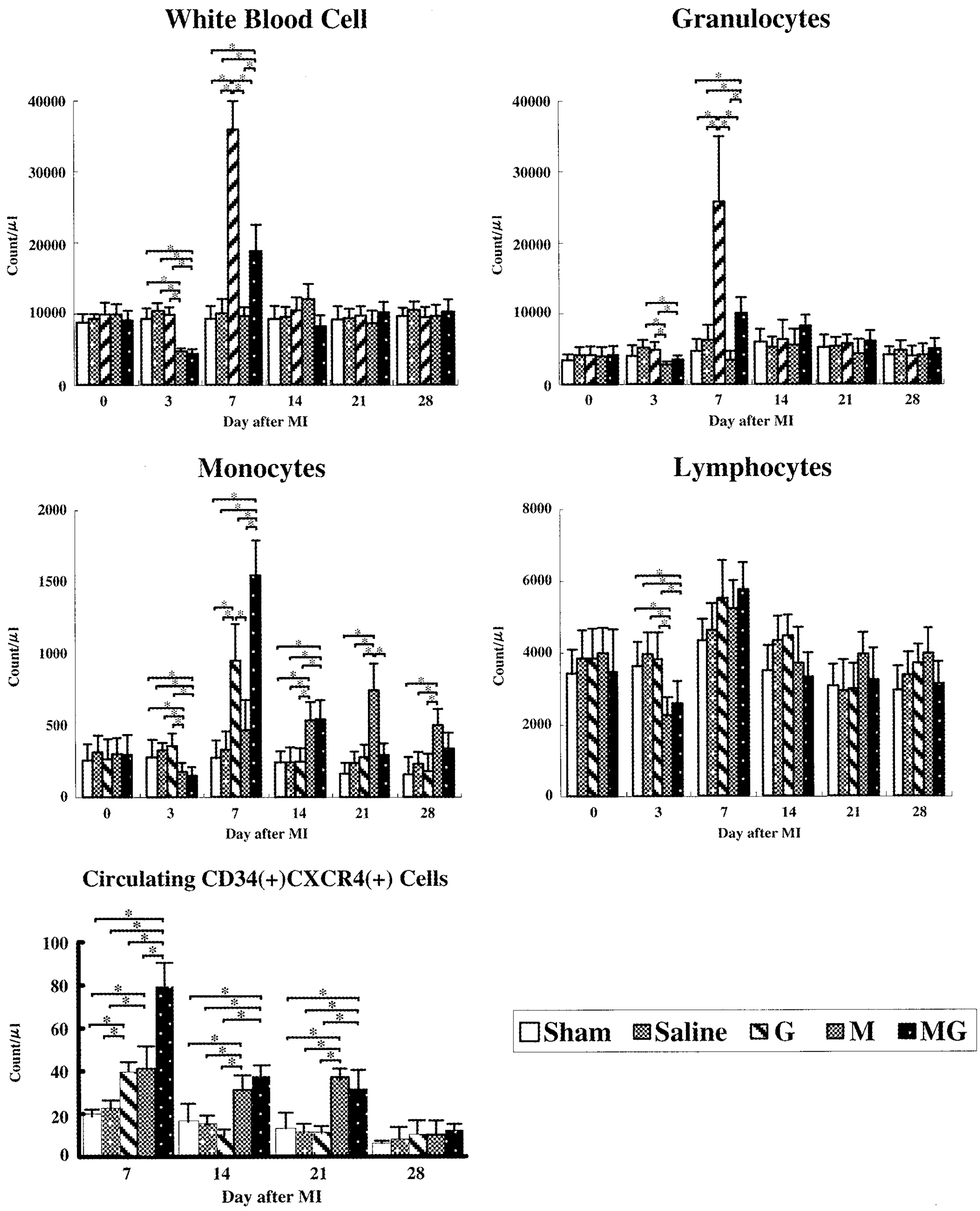

Fig 3. Peripheral blood cell counts (WBC). Granulocyte-colony stimulating factor (G-CSF) increased the number of WBCs and granulocytes 7 days after infarction, although the number of monocytes and circulating CD34+CXCR4+ cells also increased in comparison with the saline group. In contrast, G-CSF with myelosuppressives further increased the number of circulating CD34+CXCR4+ cells and monocytes in spite of the presence of leukocytosis attenuated by the preceding myelosuppression. MI, myocardial infarction.

were significantly higher in the G group than in any other group on day 7 post-MI. However, these increases were suppressed in the MG group. The numbers of monocytes and circulating CD34+CXCR4+ progenitor cells were highest in the MG group. The treatment had no effect on either red blood cells or platelets (data not shown).

General Pathology

Seven days post-MI, the infarcted areas contained granulation tissue comprised of myofibroblasts and small vessels; scar tissue comprising collagen and fatty cells was seen 28 days post-MI. As shown in Fig 4, the transverse diameters of cardiomyocytes were significantly larger in the area bordering the infarction than in remote areas, and there was no significant difference among the 4 groups. In addition, the LV weights and LV wall areas were similar in the 4 groups 28 days post-MI; however, the infarct areas and the scar areas were the smallest in the MG group, and smaller in the M and G groups than in the S group. Conversely, the surviving areas were largest in the MG group and larger in the M and G groups than in the S group. These effects were all eliminated with the administration of AMD3100.

Immunohistochemistry

The numbers of RAM 11+ macrophages and CXCR4+ cells within the infarcted areas 7 days post-MI were significantly greater in the MG group than in the M and G groups,

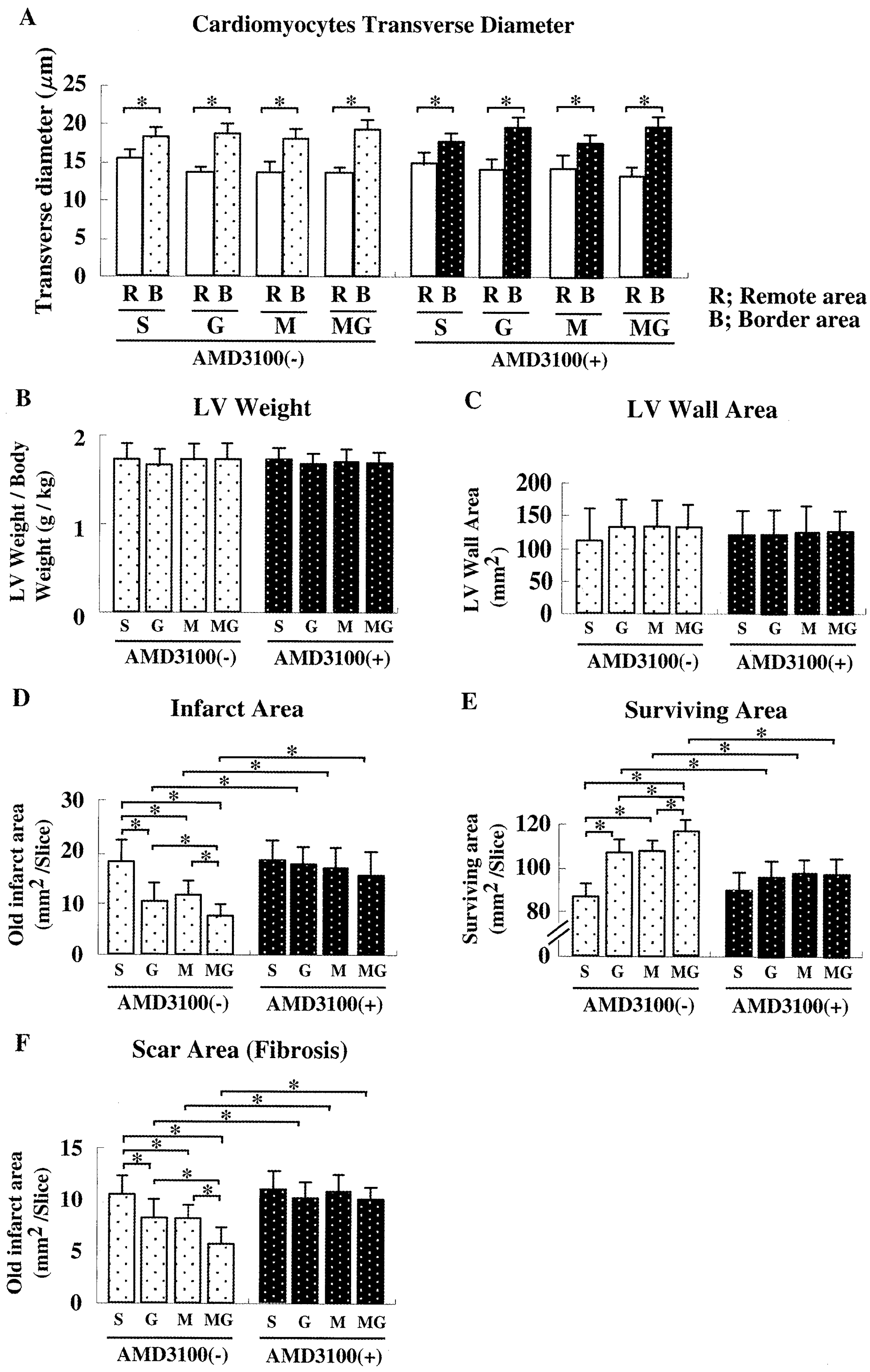

Fig 4. The transverse diameters of cardiomyocytes, left ventricular (LV) weight, LV wall area, infarct area, surviving area, and scar area 28 days post-myocardial infarction (MI). Granulocyte-colony stimulating factor (G-CSF) and/or myelosuppressives reduced the infarct areas and the scar areas, and induced a surviving myocardium, while the effect was the greatest in the G-CSF of the myelosuppressives group, and this effect was inhibited by AMD3100. There were no significant differences in LV weight, LV wall areas and cardiomyocyte transverse diameters among the groups. The border area was defined as areas of surviving myocardial tissue within 1 mm of the infarcted area.

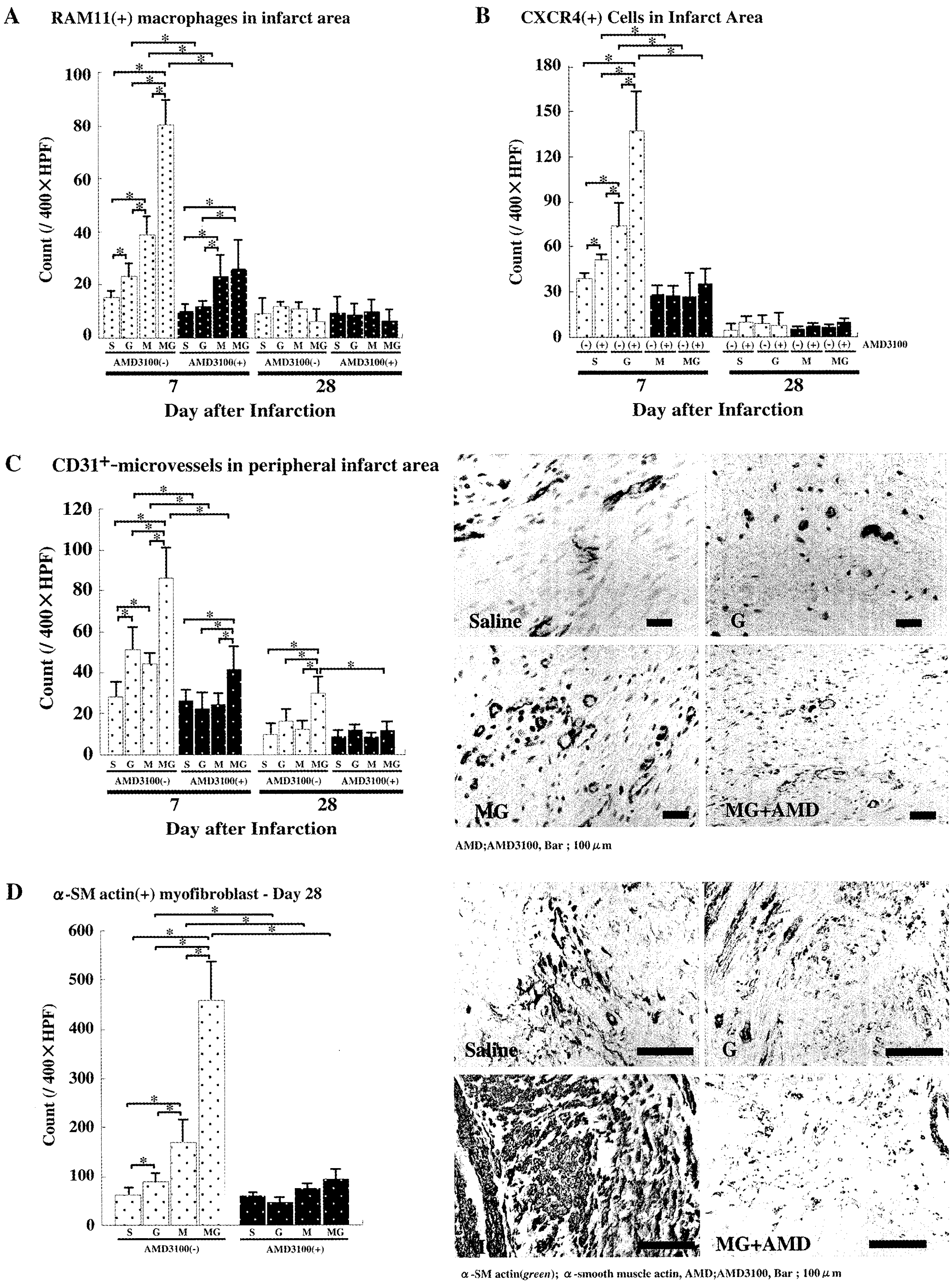

Fig 5. An immunohistochemistry in the infarcted tissues. Recruitment of RAM11+ macrophages (A) and CXCR4+ cells (B) was induced by the granulocyte-colony stimulating factor (G-CSF) and/or myelosuppressives in comparison with the saline group, while G-CSF with myelosuppressives was found to be the most effective in the cell-recruiting effect. Increased counts of α-smooth muscle (SM) actin+ myofibroblasts and CD31+ endothelial cells within infarcted areas (per high power field) was seen in the treated groups, and furthermore, the combination therapy induced the greatest proliferation of microvessels and myofibrobrasts. Interestingly, the effects were inhibited by AMD3100. HPF, high power field.

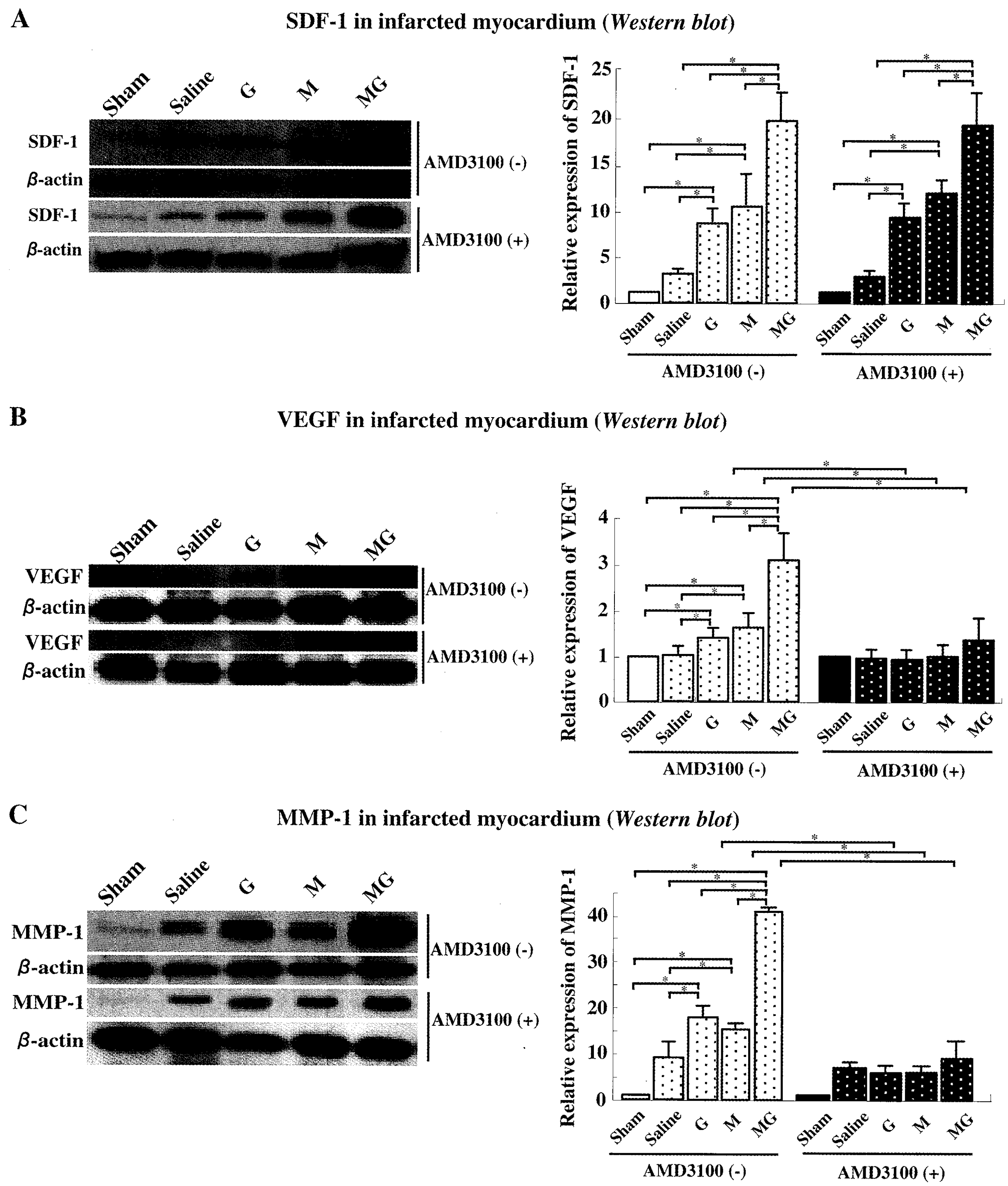

Fig 6. Western blot 7 days post-myocardial infarction. A greater upregulation of stromal cell-derived factor (SDF)-1, vascular endothelial growth factor (VEGF) and matrix-metalloproteinase (MMP)-1 in the left ventricular wall with infarction was induced by granulocyte-colony stimulating factor (G-CSF) with myelosuppressives than by any other treated groups, by G-CSF or myelosuppressives, which were all inhibited by AMD3100.

which were in turn greater than in the S group, but the increases were blocked by AMD3100 (Figs 5A,B). In addition, the numbers of CD31+ capillaries and α-smooth muscle actin+ myofibroblasts were greater in the infarcted areas of the MG group than in those of the other groups on day 28 post-MI. Again the increases were blocked by AMD3100.

Western Blotting

As shown in Fig 6, the expression of SDF-1, VEGF and MMP-1 within the infarcted LV wall was significantly greater on day 7 post-MI in the MG group than in the M and G groups, which were in turn greater than in the S group. AMD3100 inhibited the upregulation of VEGF and MMP-1, but not the upregulation of SDF-1.

BM Cell-derived Cardiomyocytes and Endothelial Cells

The ratios of DiI-labeled cardiomyocytes to total cardiomyocytes and DiI-labeled endothelial cells to total endothelial cells in the areas bordering the infarction were

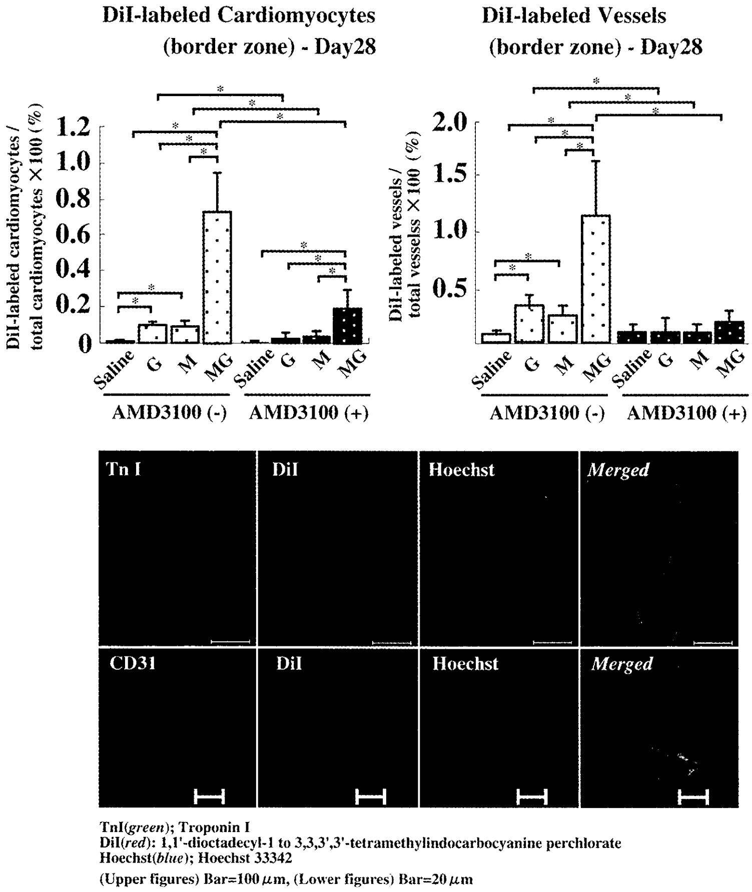

Fig 7. Bone marrow-derived mononuclear cell (BM-MNC)-derived cardiomyocytes and endothelial cells detected by confocal microscopy 28 days post-myocardial infarction. Granulocyte-colony stimulating factor (G-CSF) and/or myelosuppressives increased the number of DiI-labeled-cardiomyocytes, thus suggesting the presence of BM-MNC-derived cardiomyocytes and endothelial cells, in the area bordering infarction, and note that G-CSF with myelosuppressives most effectively increased the cell number, and the effect was attenuated by AMD3100.

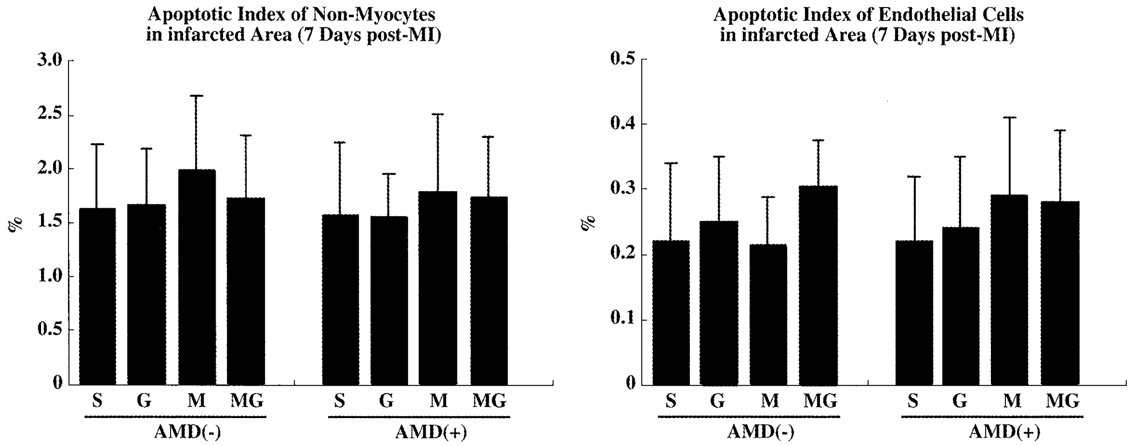

Fig 8. Apoptosis. Apoptotic indices of interstitial non-myocytes cells or endothelial cells within the infarcted area 7 days post-myocardial infarction (MI). Note that none of the therapies affected the apoptotic indices.

calculated for each group (Fig 7). Both of these ratios were significantly higher in the MG group than any of the other groups, and these increases were abolished in rabbits receiving AMD3100.

Apoptosis

Seven days post-MI, interstitial non-myocytes (eg, infiltrated inflammatory cells, endothelial cells) were often found to be TUNEL-positive; however, no TUNEL-positive cardiomyocytes were identified. As shown in the Fig 8, there were no significant differences in apoptotic indices among the treatment groups, which is consistent with our earlier findings.[13,14] In addition, co-administration of AMD3100 did not affect the incidence of apoptosis.

Discussion

Comparison of the Mechanisms Underlying the Beneficial Effects of G-CSF and Myelosuppressives

G-CSF and myelosuppressives show similar beneficial effects, as judged by their capacities to increase LVEF and diminish remodeling. The underlying mechanisms also are similar: they both reduce the size of old infarcts, increase the areas of surviving LV, increase the numbers of macrophages, CXCR4+ cells and microvessels present, up-regulate VEGF and MMP-1, and, as herein shown, increase numbers of BM-derived cardiomyocytes and endothelial cells. That these beneficial effects were all inhibited by AMD3100, a specific CXCR4 antagonist, suggests they all depend on the recruitment of BM-derived cells into the myocardium.

As compared with myelosuppressives, G-CSF acts rapidly to induce cell migration by directly affecting CXCR4/SDF-1 signaling, but its effects quickly disappear, which is also shown in the present study.[15] By contrast, myelosuppressives first affect cell-cycling BMSCs then indirectly summon quiescent ones and activate them, eventually increasing the numbers of circulating BMSCs, so that the mobilization of CD34+CXCR4+ cells into the peripheral circulation is sustained longer.[6] Consistent with those findings, we observed that the numbers of CD34+ CXCR4+ cells in the peripheral circulation was elevated for a longer period in the M group than in the G group. Nevertheless, the efficiency by myelosuppressives was similar to that by G-CSF. This may be explained by 2 following possibilities. Firstly, the fact that upregulation of SDF-1 by both myelosuppressives and G-CSF, which is required to recruit circulating BMSCs into the myocardium, was limited to 7 days post-MI; thereafter, SDF-1 was no longer expressed within the infarcted myocardium (data not shown).[9] Consequently, even if a greater number of BMSCs are mobilized into the circulation by myelosuppressives, their recruitment into the myocardium may be limited by the availability of SDF-1. Second, unlike myelosuppressives, which are superior to G-CSF in the capacities to mobilize BMSCs, G-CSF has been reported to have an additional "cardioprotective" effect against ischemia-reperfusion injury.[16,17] As a result, they might seem similar in their apparent beneficial effects.

Beneficial Effects of Post-MI Administration of G-CSF and Myelosuppressives

Previous studies have shown that post-MI administration of G-CSF or myelosuppressives improves cardiac function and diminishes LV remodeling.[1,8] In the present study, the LVEF was greater and LVEDD was smaller in rabbits receiving the combination therapy than in those receiving either myelosuppressives or G-CSF alone. This suggests that the 2 agents act synergistically to improve cardiac structure and function following infarction. Moreover, according to Cesane et al, CD34+ cells mobilized by the combination of myelosuppressives and G-CSF would be functionally different from CD34+ cells mobilized by G-CSF alone. In brief, CD34+ cells mobilized by the combination therapy have higher clonogenic activity and primitive progenitor cell content than CD34+ cells mobilized by G-CSF.[18]

The leukocyte count is reportedly an independent risk factor and a prognostic factor associated with adverse outcomes in the clinical setting after MI because leukocytosis can lead to multiple adverse responses, including thrombosis among others.[19] Post-MI administration of G-CSF causes a remarkable degree of leukocytosis, producing leukocyte counts of up to 40,000/μl, which could be a considerate issue in clinical settings. Likewise, in the present study, G-CSF treatment induced marked increases in the leukocyte and granulocyte counts by day 7 post-MI. In contrast, myelosuppressives reduced the number of granulocytes, and in combination with G-CSF, they apparently inhibited the increases in the leukocyte and granulocyte counts seen with G-CSF alone.

Possible Mechanism for the Improvement of Cardiac Structure and Function

We suggest 2 possible mechanisms underlying the improvement in cardiac structure and function elicited by G-CSF and myelosuppressives. First, the improvement in LV function may reflect repair-relating paracrine effects mediated by the cells recruited into the infarcted myocardium. The smaller scar tissue areas seen in the MG group likely contributed to the improved cardiac function and diminished remodeling. This would be explained by the significantly enhanced expression of MMP-1, which likely catalyzes the breakdown of collagen during the scar-formation process. Previous studies reported that an MMP family was increased in the post-MI heart failure models with permanent occlusion and large infarction, and the inhibitors beneficially affected cardiac remodeling and function, suggesting that an increase of MMP has a aggravating effect on heart failure via collagen degradation.[20,21] In such cases with large infarction, collagen accumulation plays a critical role in preventing ventricular remodeling. In contrast, in the model of the present study, with moderate-sized infarction by a transient ischemia and reperfusion protocol, an excessive amount of fibrosis without contractility or relaxation would accelerate cardiac remodeling and decrease cardiac function, as seen in ischemic or idiopathic dilated cardiomyopathy, and accordingly, to maintain fibrosis to an adequate extent by an anti-fibrotic effect of MMP would be beneficial in preventing ventricular remodeling.[22,23] Similarly, levels of the angiogenic growth factor VEGF were highest in the MG group, which may be related to the observed increase of microvessels. Marked proliferation of myofibroblasts containing large amounts of actin was also observed within the infarcted areas in the MG group. By protecting against wall stress, these cells also may play an important role in preventing ventricular remodeling.[24]

The second is regeneration of myocardial tissue. The surviving LV area was greatest, and the numbers of BM-derived cardiomyocyte-like cells within infarcted tissues were highest in the MG group, as a result of induced recruitment of CD34+CXCR4+ cells, which is consistent with a previous report of dose-dependent CD34+-cell contribution to regeneration of infarcted myocardium.[25] That all of these beneficial effects were blocked by AMD3100 is indicative of the importance of the paracrine effects and new tissue formation mediated by mobilized CXCR4+ cells within infarcted myocardial tissues. However, it is still unclear, as a limitation of the present study, whether the new myocytes and endothelial cells were formed by cell differentiation or by cell fusion.

A recent report has shown that G-CSF directly exerts an anti-apoptotic effect on myocardium following ischemia.[26] In the present study, nevertheless, we detected no TUNEL-positive cardiomyocytes in hearts from any of the groups, but only TUNEL-positive non-myocytes, including infiltrated inflammatory cells, endothelial cells and other interstitial cells, were without significant difference in all of the groups, suggesting that the therapeutic effects of G-CSF and myelosuppressives are unrelated to apoptosis.

No Serious Adverse Effect of Myelosuppressives

The peripheral leukocyte counts were transiently reduced by myelosuppressives to approximately 5,000/μl on day 3 post-MI after their administration, which was half the control level seen on day 0, and completely recovered by day 7. On days 7 and 28 post-MI, there was no difference in their survival or histological evidence of infectious disease in the hearts or lungs of the rabbits receiving myelosuppressives, as compared with rabbits not receiving myelosuppressives (data are not shown).

Clinical Implications

The efficacy of G-CSF alone for the treatment of MI remains controversial, as shown in the negative results of a recent double-blind controlled clinical trial.[5] As herein shown, the beneficial effects of G-CSF alone were significantly smaller than those elicited when G-CSF was administered in combination with myelosuppressives, which indicates there is room to improve G-CSF therapy. Accordingly, a modified G-CSF therapy, such as the one examined here, might be a more effective, novel strategy for enhancing post-MI myocardial repair in the clinical setting than G-CSF alone. In fact, nevertheless, myelosupressives such as 5-fluorouracil and cyclophosphamide are potentially cardiotoxic. Five-fluorouracil gives rise to spasms of the coronary arteries, rhythm disturbance and myocardial and endothelial inflammation underlying their apoptosis at an acute phase of treatment, although at a higher dosage than in the present study.[27] Similarly, a higher dose of cyclophosphamide is toxic for endothelium and myocytes, and causes interstitial hemorrhage and edema.[28] Although the dosage of the present experimental setting did not lead to any severe adverse effects, we need to monitor hemodynamics following treatment of the agents in the clinical setting closely. In conclusion, the combined administration of G-CSF and myelosuppressives following MI improves cardiac function and diminishes LV remodeling via increases in circulating CXCR4+ cells including CD34+ cells and repair-related inflammatory cells such as monocytes/macrophages, and may be a useful new therapy that overcomes the insufficiency seen with G-CSF alone.

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