Granulocyte colony-stimulating factor attenuates early ventricular expansion after experimental myocardial infarction

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Abstract

Objective: In the early phase after transmural myocardial infarction (MI), the infarcted myocardium undergoes replacement by scar tissue, which is essential for preserving the structural integrity of the infarcted tissue. Transforming growth factor (TGF)-β1, which is known as a fibrotic cytokine, plays a pivotal role in the reparative fibrosis after MI. It is reported that granulocyte colony-stimulating factor (G-CSF) can accelerate wound healing. The aim of our study was to investigate the effect of G-CSF on early ventricular expansion after MI.

Methods: MI was induced by ligation of the left coronary artery in male Wistar rats. G-CSF (20 μg/kg/day, MI-GCSF) or saline (MI-saline) was injected subcutaneously 3 h after MI and every 24 h thereafter for 7 days. Hemodynamic and echocardiographic studies were performed at 14 days. Expression of TGF-β1 and procollagen type I and type III mRNA in both the infarcted and noninfarcted areas was studied by quantitative RT-PCR at 1, 3, 7, and 14 days after MI. Histological studies were performed at 7 days.

Results: MI-GCSF had higher LV max dP/dt, lower LV end-diastolic pressure, and smaller LV end-diastolic and end-systolic dimensions compared to MI-saline. Infarct size was not different between MI-GCSF and MI-saline. Expression of TGF-β1 mRNA in the infarcted area at 3 days was significantly higher in MI-GCSF than in MI-saline. Expression of procollagen type I and type III mRNA in the infarcted area at 3 days was higher in MI-GCSF compared to MI-saline, and the peak mRNA levels were earlier in MI-GCSF. In the noninfarcted area, there was no difference in TGF-β1 mRNA expression between MI-GCSF and MI-saline. Histologically, collagen accumulation in the infarcted area at 7 days was more prominent in MI-GCSF than in MI-saline.

Conclusion: G-CSF treatment improves early post-infarct ventricular expansion through promotion of reparative collagen synthesis in the infarcted area, suggesting some beneficial effect of G-CSF on the infarct healing process.

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Keywords: Cytokines; Fibrosis; Growth factors; Infarction; Remodeling

1. Introduction

As a consequence of large myocardial infarction (MI), the process of complex architectural myocardial alterations, referred to as ventricular remodeling, is initiated. Acute myocyte loss and breakdown of extracellular matrix induce early ventricular expansion, which is associated with deleterious cardiac function in the late phase [1,2]. Replacement by collagen fibers is essential in preserving the structural integrity of infarcted tissue and protects against increased dilatation of the left ventricle (LV) [3–5]. Cytokines play major roles in this healing process, among which transforming growth factor (TGF)-β1, a fibrotic cytokine, is extremely important in reparative fibrosis [6–8]. It is well known that infarct size and transmurality are determinants of the ventricular remodeling process, as there...
is evidence that early reperfusion of the infarcted artery limits the extent of myocardial necrosis and attenuates ventricular enlargement [1]. The infarct repair process, involving inflammation and collagen synthesis, is another important factor that affects ventricular remodeling. While an excessive inflammatory reaction in the infarcted myocardium is associated with poor clinical outcomes, such as ventricular rupture or congestive heart failure [9–11], anti-inflammatory therapy after MI using corticosteroid leads to catastrophic results, by delaying collagen accumulation and scar formation, in both clinical and experimental settings [12–14]. These findings suggest that inflammation is essential for the appropriate infarct healing process.

Granulocyte colony-stimulating factor (G-CSF), a 20-kDa glycoprotein, is known to induce granulopoiesis [15]. It was reported that G-CSF in combination with stem cell factor improved ventricular remodeling after MI through myocyte regeneration by way of transdifferentiation of bone marrow stem cells [16]. However, recent reports suggested that hematopoietic stem cells do not transdifferentiate into cardiac myocytes after MI [17,18]. G-CSF has been described as having immunoregulatory properties in addition to inducing cell differentiation [19]. This cytokine can induce earlier healing in cutaneous wounds by acting on the inflammatory system [20,21]. Therefore, especially in the environment of the early phase of MI, where intense inflammation occurs, it is possible that G-CSF affects the healing process by modulating the inflammatory reaction and collagen synthesis.

We hypothesized that administration of G-CSF after MI attenuates early ventricular expansion through accelerating reparative fibrosis in the infarcted area. Accordingly, we investigated whether G-CSF administration affects post-MI ventricular function and whether such effects are associated with myocardial fibrotic cytokine expression and collagen synthesis.

2. Methods

All procedures were performed in accordance with the Keio University animal care guidelines, which conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. Animals and surgical procedure

Left ventricular MI was created in 9-week-old male Wistar rats (Sankyo Laboratory Service, Tokyo, Japan) weighing 200–230 g, by left coronary artery ligation as previously described [22]. Briefly, rats were anesthetized with intraperitoneal pentobarbital, intubated, and then ventilated with positive end-expiratory pressure. After left thoracotomy and pericardiotomy, the left coronary artery was permanently ligated 2 mm from the origin with a 6-0 silk suture, and then the chest was closed. Limb lead ECG was constantly monitored throughout the procedure, and the induction of MI was identified by ST segment elevation in lead II (Fig. 1). Coronary ligation was performed in 162 rats. The same procedure was performed for sham-operated control (n=11), but the coronary ligature was left untied. MI rats surviving the operation for 3 h (n=131) were randomly assigned to two groups: (1) recombinant human G-CSF (20 μg/kg/day, Kyowa Hakko Kogyo, Tokyo, Japan) administered subcutaneously for 7 days (MI-GCSF, n=63), and (2) placebo-treated control (MI-saline, n=68). Rats were housed under standardized conditions with free access to standard diet and drinking water. Eighty-seven rats (MI-saline, n=44; MI-GCSF, n=43) survived and were sacrificed 1, 3, 7, and 14 days after surgery according to the study protocol.

2.2. Peripheral neutrophil count

A 0.5 ml blood sample was serially (on days 1, 3, 7, and 14) drawn from the tail vein to measure peripheral neutrophil count. Total white blood cell count was determined with an automated hematology analyzer (XE-2100, Sysmex, Kobe, Japan). Peripheral blood smears were stained with Wright–Giemsa, and neutrophil count was obtained after differential cell counting of white blood cells performed under a light microscope on 200 stained cells. Five animals in each group were studied.

![Fig. 1. Representative ECG trace in lead II, showing ST segment elevation as index of ischemia induced by left coronary artery ligation.](image-url)
2.3. Echocardiographic, hemodynamic and heart weight measurements

Animals (sham-operated rats, \( n = 7 \); MI-saline, \( n = 10 \); MI-GCSF, \( n = 9 \)) were lightly anesthetized with intraperitoneal pentobarbital 2 weeks after MI. Two-dimensional and M-mode echocardiographic (8.5-MHz linear transducer; EnVisor C, Philips Medical Systems, Andover, MA, USA) images were obtained to assess LV end-diastolic dimension (LVEDD) and LV end-systolic dimension (LVESD) at the mid-papillary muscle level and LV fractional shortening (FS) was calculated: \( \text{FS} (%) = \left( \frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD}} \right) \times 100 \). After echocardiography, a miniature pressure transducer (SPC-320, Millar Instruments, Houston, TX, USA) was placed in the right carotid artery and then advanced retrogradely into the LV. Electrocardiogram and pressure were recorded continuously on a PC using a data acquisition system (PowerLab, ADInstruments, Colorado Springs, CO, USA). LV pressure, rate of rise in LV pressure (\( \frac{dP}{dt} \)), and heart rate were obtained. LV systolic pressure, maximum and minimum values of \( \frac{dP}{dt} \), and heart rate were determined by averaging the data of 10 consecutive beats. The hearts were excised, divided into LV and RV, and each was weighed separately. The investigators who performed these procedures were blinded regarding sham vs. MI and saline vs. G-CSF.

2.4. Total RNA and protein isolation

At 1, 3, 7, and 14 days after surgery, rats were sacrificed for RNA and protein analysis. Twenty-four animals in each group (six each at 1, 3, 7, and 14 days post-MI) were studied. After the hearts were rapidly excised, the RV free wall was trimmed away, and the infarcted area was cut from the noninfarcted region. The infarct border was included in the infarcted area. Both infarcted and noninfarcted tissues were snap frozen in liquid nitrogen, and then preserved at −80 °C. LV of sham-operated rats (on day 14, \( n = 4 \)) was collected for control.

Total RNA was isolated by a modification of the acid guanidinium thiocyanate and phenol/chloroform extraction method. After homogenizing the heart tissues with a Polytron homogenizer in Trizol-Reagent (Invitrogen, Carlsbad, CA, USA), total RNA was extracted with chloroform and samples were centrifuged at 12,000 \( \times g \) for 15 min at 4 °C. RNA was precipitated by addition of isopropanol, and the pellet was dissolved in diethyl pyrocarbonate water. Total RNA concentration was determined by spectrophotometric analysis at 260 nm. For Western blot analysis, frozen tissue was homogenized in PBS buffer containing 0.5% Triton X-100, 0.01% sodium azide, and protease inhibitors. After centrifugation at 16,000 \( \times g \) for 30 min at 4 °C, the supernatants were collected. Protein concentrations were measured using Coomassie protein assay reagent (Pierce Biotechnology, Rockford, IL, USA) based on the Bradford assay.

2.5. Real-time quantitative RT-PCR

Real-time quantitative RT-PCR of each sample was carried out with a TaqMan RNA PCR kit and ABI Prism™ 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. Sequence-specific PCR primers and TaqMan probes for TGF-\( \beta_1 \), procollagen type I and type III, interleukin (IL)-6, and tumor necrosis factor (TNF)-\( \alpha \) were designed using Primer Express software version 1.7 (Applied Biosystems); see Table 1 for details. Reverse transcription was performed at 48 °C for 30 min using TaqMan Reverse Transcription reagents (Applied Biosystems). PCR reactions were performed in triplicate for 10

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession no.</th>
<th>Primer sets and TaqMan probes used for real-time quantitative RT-PCR analysis</th>
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<tr>
<td>TGF-( \beta_1 )</td>
<td>NM_021578</td>
<td>(F) GGCACCATCCATGACATGAA (R) CAGGTTGTGAGCCCCCTTCCA (T) FAM-CCTTCCTGCTCCTCATGGCCACC-TAMRA</td>
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<td>Procollagen type I</td>
<td>Z78279</td>
<td>(F) CCAGTTCTGAGTAGGAGCGA (R) AGGTGATGTTCTGGG (T) FAM-CCTGGGCGCTGTAGTCCACCGA-TAMRA</td>
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<tr>
<td>Procollagen type III</td>
<td>X70369</td>
<td>(F) CAGCTGCGCTCTCTGACACTT (R) GCTGTGTTGCACTGTTGATGTAATGT (T) FAM-TTTCCAGGGCCGCTCCACG-TAMRA</td>
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<td>IL-6</td>
<td>M26744</td>
<td>(F) AATGGAGAAGGTAGTACAGAAAGG (R) CCGAGTACAGACTCTATGACCTTGTAG (T) FAM-TGGCTAAAGGACCAACAGACTCAACTCA-TAMRA</td>
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<tr>
<td>TNF-( \alpha )</td>
<td>NM_012675</td>
<td>(F) TGGGCTCCCCCTCATGCTGT (R) TGGGCTAGGAGAGTCACTGTCACAATCAATAG (T) FAM-TGGCTACAGACTCACTCAACTCA-TAMRA</td>
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<tr>
<td>GAPDH</td>
<td>AF106860</td>
<td>(F) AACTCCCTCAAGATTGTCAGCAA (R) GTGGTCTATGAGCCCCCTTCCA (T) FAM-CTGCACCCACCAACTGTCCTTACCGCC-TAMRA</td>
</tr>
</tbody>
</table>

Accession no., GenBank accession number; (F), forward primer; (R), reverse primer; (T), TaqMan probe.
min at 95 °C for DNA polymerase activation, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C with a final 25 °C hold, using a PCR reaction mixture containing 25 μl Mastermix (TaqMan, Applied Biosystems), 900 nM forward and reverse primers, 200 nM TaqMan probe, and 200 ng cDNA in a total volume of 50 μl. The threshold number of cycles (CT) was calculated by determining the point at which the fluorescence intensity was 10 times larger than the standard deviation of the baseline fluorescence. For each sample, CT values were subtracted from that of the housekeeping gene, GAPDH, to generate the CT value.

2.6. Western blot analysis

Western blot analysis was carried out as previously described [22]. Equal amounts of 50 μg protein were electrophoresed on 10% SDS polyacrylamide gels (150 V, 60 min). Proteins were electroblotted onto nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ, USA) for 60 min at 100 V. After blocking with 5% non-fat dried milk in PBS for 60 min, the membranes were incubated with rabbit polyclonal anti-rat TGF-β1 antibody (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 60 min, followed by exposure to horseradish peroxidase-linked anti-rabbit immunoglobulin (1:5000, Amersham) for 30 min. The immunoblots were developed by enhanced chemilumino-fluorescence method. The signals were quantified by densitometry (GS-800, Bio-Rad, Hercules, CA, USA).

2.7. Infarct size and expansion index measurement

At 7 days after MI, 30 mM potassium chloride was injected intravenously to cause cardiac arrest in diastole. The hearts (n=5 in each group) were excised, fixed with 10% formaldehyde, and embedded in paraffin for histological analysis, including measurement of infarct size, expansion index, collagen volume fraction, and infiltrating neutrophils. After fixation, the hearts were cut transversely from apex to base into four equal slices. The sections were stained with hematoxylin–eosin and Masson’s trichrome. The boundary lengths of the infarcted and noninfarcted endocardial and epicardial surfaces of all slices were traced using Scion Image software (Scion, Frederick, MD, USA), and the infarct size was determined as the percentage of infarcted epicardium and endocardium of the LV [23]. The area of the LV cavity and total LV, and thickness of the septum and scar in sections at the papillary muscle level were measured, and then the expansion index was calculated as previously described [24]: Expansion Index=(LV Cavity Area/Total LV Area)×(Noninfarcted Septal Thickness/Scar Thickness). The investigators who performed the measurements of infarct size, expansion index, collagen volume fraction, and infiltrating neutrophil count were blinded as to MI-saline vs. MI-GCSF.

2.8. Collagen volume fraction

To analyze collagen accumulation, the sections were stained with picrosirius red. Deparaffinized sections were incubated with picrosirius red (0.1%) in saturated picric acid solution for 90 min. Sixteen separate areas of high power fields in each section were visualized under polarized light and photographed with the same exposure time. The collagen volume fraction (CVF) in the infarcted and noninfarcted area was calculated as the percentage of stained tissue in the sum of muscle area and connective tissue [25] by densitometric method using Scion Image software (Scion).

2.9. Immunohistochemistry

Immunohistochemical studies were performed by immunoperoxidase methods. Paraffin-embedded specimens were

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=7)</th>
<th>MI-saline (n=10)</th>
<th>MI-GCSF (n=9)</th>
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<tr>
<td>LVW/BW, mg/g</td>
<td>1.92±0.09</td>
<td>2.23±0.05*</td>
<td>2.25±0.05*</td>
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<tr>
<td>RVW/BW, mg/g</td>
<td>0.55±0.04</td>
<td>0.83±0.05*</td>
<td>0.76±0.03*</td>
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<td>LVEDD, mm</td>
<td>6.3±0.1</td>
<td>8.1±0.1*</td>
<td>7.8±0.1*</td>
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<tr>
<td>LVEDS, mm</td>
<td>3.9±0.2</td>
<td>7.1±0.1*</td>
<td>6.6±0.1*</td>
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<tr>
<td>FS, %</td>
<td>37.8±2.3</td>
<td>12.1±0.5*</td>
<td>15.5±0.7*</td>
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<tr>
<td>HR, bpm</td>
<td>411±12</td>
<td>372±15</td>
<td>391±9</td>
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<tr>
<td>LVSP, mmHg</td>
<td>120±2</td>
<td>94±3*</td>
<td>97±4*</td>
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<td>dP/dtmax, mmHg/s</td>
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<td>4445±252*</td>
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<tr>
<td>−dP/dtmax, mmHg/s</td>
<td>−7702±1017</td>
<td>−3127±267*</td>
<td>−3653±167*</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>3.3±0.6</td>
<td>12.6±0.7*</td>
<td>10.0±0.6*</td>
</tr>
</tbody>
</table>

LVW, left ventricular weight; RVW, right ventricular weight; BW, body weight; LVEDD, left ventricular end-diastolic dimension; LVEDS, left ventricular end-systolic dimension; FS, fractional shortening; HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure. Values are mean±S.E.M.

* P<0.05 vs. sham.
† P<0.05 vs. MI-saline.
cut into 6-μm-thick sections and mounted on slides, followed by deparaffinization in xylene and dehydration through a graded series of ethanol concentrations. After washing the sections, they were incubated with polyclonal rabbit anti-human myeloperoxidase antibody (MPO, DAKO, Glostrup, Denmark) for 60 min to identify the infiltration of granulocytes. The sections were then treated with peroxidase-conjugated anti-rabbit antibody (Envison kit, DAKO) for 30 min. The reaction was visualized with 3,3′-diamino-benzidine tetrahydrochloride (DAB) as a substrate (DAKO). Neutrophil quantitation was performed by counting the number of MPO-positive cells in 10 grid fields with a total area of 0.1 mm².

2.10. Statistics

All data were expressed as mean±S.E.M. Comparisons between two groups were performed using Student’s t-test.

![Graphs](image-url)

Fig. 3. Real-time quantitative RT-PCR analyses of TGF-β (infarcted area) in (A) and noninfarcted (B) myocardium, and of procollagen type I (C), type III (D), IL-6 (E), and TNF-α (F) gene expression in infarcted myocardium. Bars represent mRNA levels relative to GAPDH mRNA level for MI-saline (open bars) and MI-GCSF (closed bars). Data are presented as the ratio to sham. Values are mean±S.E.M. *P<0.05 vs. MI-saline.
When more than two groups were analyzed, one-way analysis of variance followed by Scheffe’s post hoc test was employed. A value of $P<0.05$ was considered significant.

3. Results

3.1. Peripheral neutrophil count

Neutrophil counts in the drawn peripheral blood are shown in Fig. 2. After MI was induced, the peripheral neutrophil count in MI-saline was increased on day 1, and then decreased. The neutrophil count in MI-GCSF showed a greater increase than that in MI-saline on day 1, was elevated until day 7, and decreased thereafter. Thus, there was a prolonged augmented response of peripheral neutrophil count to G-CSF after MI induction.

3.2. LV functional analysis

Left and right ventricular weight per body weight, heart rate and left ventricular systolic pressure in both the MI-GCSF and MI-saline groups did not differ significantly on day 14. Left ventricular end-diastolic pressure (LVEDP) was lower in MI-GCSF than in MI-saline (Table 2). Maximum $dP/dt$ was higher in MI-GCSF than in MI-saline. MI-GCSF had significantly lower LVEDD and LVESD, and higher FS compared to MI-saline (Table 2).

3.3. Myocardial RNA expression

mRNA expression of TGF-$\beta_1$ in the infarcted area of MI-saline was elevated on day 1, peaked on day 3 up to approximately 7.5-fold compared to that of sham-operated rats, and then slowly declined. TGF-$\beta_1$ mRNA expression in the infarced area of MI-GCSF also showed a peak level on day 3, the same as MI-saline, whereas the maximum value of MI-GCSF was significantly higher than that of MI-saline (Fig. 3A). In the noninfarcted area, mRNA expression level of TGF-$\beta_1$ did not differ between the two groups during the entire study period (Fig. 3B). Procollagen type I and type III mRNA expression levels in the infarcted area of MI-GCSF reached their peaks on day 3, whereas those of MI-saline increased gradually, showing peaks on day 7, and then decreased. Their peak expression was earlier and higher in MI-GCSF compared with MI-saline (Fig. 3C,D). No difference in IL-6 and TNF-$\alpha$ mRNA expression levels in the infarcted area was observed between MI-saline and MI-GCSF at each time point (Fig. 3E,F).

3.4. Western blot analysis

Levels of TGF-$\beta_1$ protein in the infarcted area at different time point were analyzed by Western blotting. We mainly observed bands corresponding to the latent form of TGF-$\beta_1$ at 50 kDa in each lane (Fig. 4). There was an increase in TGF-$\beta_1$ protein in the infarcted area in both the MI-GCSF and MI-saline groups compared to sham at each time point. Highest TGF-$\beta_1$ expression was noted on day 3 in each group, and MI-GCSF showed significantly higher expression of TGF-$\beta_1$ than MI-saline.

3.5. Infarct size and morphology

Both MI-GCSF and MI-saline groups had similar infarct size (49±3% vs. 50±2%, $P=NS$, Fig. 5). However, MI-GCSF showed less chamber dilatation and less ventricular hypertrophy in the noninfarcted area (Fig. 6A,B). The expansion index was significantly lower in MI-GCSF than in MI-saline (1.51±0.09 vs. 1.81±0.07, $P<0.05$, Fig. 5). High-power field of Masson’s trichrome-stained sections showed more collagen accumulation in the infarcted area in MI-GCSF than in MI-saline (Fig. 6C,D).

3.6. CVF and neutrophil infiltration

Representative picrosirius red-stained sections with polarized light are shown in Fig. 7A (MI-saline) and B (MI-GCSF). Quantitative analysis indicated that CVF in the
Infarcted area was significantly greater in MI-GCSF compared to that in MI-saline (24±2% vs. 18±2%, \( P<0.05 \), Fig. 7C), although CVF in the noninfarcted area did not differ between the two groups (3.2±0.5% vs. 3.5±0.6%, \( P=\text{NS} \), Fig. 7D). MPO staining showed that infiltrating neutrophil count in the infarcted myocardium

Fig. 6. Representative Masson's trichrome-stained cross sections of infarcted hearts and magnified images of infarcted area (A, C: MI-saline; B, D: MI-GCSF). Scale bars represent 1 mm (A, B) and 50 \( \mu \text{m} \) (C, D).

Fig. 7. Representative picrosirius red-stained sections of infarcted area in MI-saline (A) and MI-GCSF (B) groups, showing characteristic bright yellow of collagen fibrils under polarized light. Scale bar indicates 100 \( \mu \text{m} \). Collagen volume fraction of infarcted (C) and noninfarcted (D) myocardium. Values are mean±S.E.M. *\( P<0.05 \) vs. MI-saline.
was not significantly different between MI-GCSF and MI-saline (33±3 vs. 35±3 cells/mm², P=NS).

4. Discussion

This study demonstrated that G-CSF administration after MI attenuates post-infarct ventricular expansion, in association with increased TGF-β1 and collagen expression in the infarcted area during the early phase of MI. Collagen analysis showed more markedly enhanced collagen deposition in G-CSF-treated MI than in control MI. G-CSF administration after MI might be beneficial to attenuate infarct expansion through accelerating reparative fibrosis in the infarcted area.

4.1. Effects of G-CSF on post-MI remodeling

Since the hypothesis that bone marrow hematopoietic stem cells regenerate infarcted myocardium was proposed, G-CSF has received attention as a cytokine that mobilizes bone marrow stem cells into the peripheral circulation [26,27]. Previously, G-CSF administration in combination with stem cell factor was reported to have a beneficial effect on LV remodeling after MI by inducing the differentiation of mobilized hematopoietic stem cells into cardiac myocytes [16]. Contrary to this concept, recent studies revealed that hematopoietic stem cells do not transdifferentiate into cardiac myocytes, but develop into mature hematopoietic cells under ischemic conditions [17,18,28]. Our data, showing no difference in infarct size after G-CSF treatment, appear to be consistent with their data. Another recent report showed an increase in neovascularization and a decrease in apoptosis in the border zone, partly explaining the beneficial effect of cytokine therapy [29]. However, in the permanent coronary occlusion model with a large MI, salvage of the border zone could not fully prevent infarct expansion. Shortly after coronary ligation, inflammatory cells such as neutrophils, monocytes and macrophages infiltrate, and then the necrotic myocytes in the injured myocardium are replaced by collagen fibers [23]. This process uniformly occurs in the whole infarcted area, and determines the degree of early infarct expansion. It is likely that the potential benefit of cytokine therapy might be derived from its impact on the repair process within the damaged tissue.

Our current study suggested that accelerated collagen accumulation caused by G-CSF was associated with better LV function after MI. Collagen synthesis, which is promoted by TGF-β1, has different significance in infarcted and noninfarcted areas [7,30–33]. Although inappropriately deposited collagen fibers in noninfarcted areas during the late phase of MI would lead to increased wall stiffness and decreased contractility of the LV, the formation of a collagen network as a replacement for necrotic myocardium may be an essential process. The accumulation of collagen fibers, which is known as reparative fibrosis, may reinforce the flail myocardium in order to yield stress tolerance and limit ventricular expansion. In an experimental model using β2-adrenergic receptor transgenic mice, the resultant increase of collagen content in the infarcted area was associated with a lower risk of cardiac rupture and less infarct expansion during the acute phase of MI [34]. In other studies using osteopontin knockout mice or rats with ETα receptor blockade, decreased collagen content in the infarcted area was associated with enhanced infarct expansion, suggesting the significance of collagen synthesis in post-MI remodeling [35,36]. Among fibrillar collagens, type I and type III are the major components in the MI heart. A previous study showed that mRNA levels of these collagens were increased around day 2, reaching a peak level on day 7 [33]. Our data, in which expression of procollagen type I and type III mRNA in the infarcted area was upregulated as early as 3 days after MI, suggested promotion of reparative fibrosis by G-CSF treatment. The enhanced TGF-β1 mRNA expression level in the early phase of MI by G-CSF treatment might contribute to early collagen synthesis in fibroblasts. Moreover, the mRNA levels of procollagens in the noninfarcted area did not differ between MI-saline and MI-GCSF. These findings might indicate that, at least within 2 weeks, reactive fibrosis in the noninfarcted myocardium was not promoted by G-CSF.

In our experimental system, we mainly observed bands corresponding to 50 kDa in immunoblotting for TGF-β1. Presumably the bands represent the 50 kDa small latent TGF-β1 complex, which consists of mature TGF-β1 and latency associated protein, as previously described [37,38]. TGF-β1 is secreted in a latent form within the extracellular matrix, and is converted to biologically active TGF-β1 by proteases under tight regulation. It was reported that the 50 kDa small latent complex of TGF-β1 possesses bioactivity [38], and is essential for regulation of the bioavailability of this cytokine [39]. Hence, the increase in expression of the latent TGF-β1 complex by G-CSF in our experiment would reflect, to a certain extent, the augmented activity of TGF-β1, which stimulates fibroblast proliferation and promotes the synthesis of extracellular matrix components.

One possible mechanism responsible for the greater collagen content in the G-CSF-treated infarcted heart appears to be the property of this cytokine to modulate the inflammatory reaction. It is recognized that the inflammatory process is essential for ventricular remodeling. We have reported that an extreme inflammatory response after MI was related to a poor clinical outcome such as ventricular rupture or congestive heart failure [9,10]. However, when inflammation is completely suppressed by corticosteroid, reparative fibrosis contributing to cardiac geometric maintenance would be delayed disadvantageously [12–14,40]. There have been several lines of evidence that G-CSF modulates the inflammatory response and promotes the repair process after tissue damage. Administration of G-CSF resulted in facilitated healing of diabetic foot ulcers and doxorubicin-induced skin wounds, and enhanced early
endothelialization in implanted vascular grafts [20,41,42]. Besides, G-CSF has a profibrotic property, enhancing the expression of TGF-β1 in peripheral inflammatory cells [43,44]. In bleomycin-induced acute lung injury rats, G-CSF exacerbated pulmonary fibrosis [45]. Therefore, the profibrotic character of this cytokine may be the driving force to accelerate wound healing.

The effect of G-CSF on circulating cytokine levels should also be considered. In addition to increasing the number of neutrophils, G-CSF alters the production of other cytokines such as TNF-α [46]. It is possible that treatment with G-CSF might affect post-MI remodeling through altered systemic cytokine profiles. Nevertheless, serum cytokine levels including those of TNF-α, IL-6, and TGF-β1 were not significantly changed in our experimental setting (data not shown). Local rather than systemic effects on cytokine production would be plausible as mechanisms of the improved post-MI remodeling by G-CSF treatment.

G-CSF receptors have been identified not exclusively on neutrophils lineage cells, but also on monocytes, macrophages, and lymphocytes [47–50]. G-CSF stimulates a variety of different cellular activities depending on the cells to which they bind. Its effect on the repair process may be mediated through a synergistic effect on local immunological effector cells and the mediators they produce. During the early phase of MI, G-CSF may act on the infiltrating cells and promote the secretion of cytokines including TGF-β1 predominantly in the infarcted area where the inflammatory cells concentrate. It is possible that G-CSF may play a role to preserve structural integrity through collagen synthesis and accumulation.

4.2. Limitations

Our current experiments were performed in rats. Since differences in the responsiveness to G-CSF between species have been described [51], caution is needed when applying these results to practical use. The dose of injected G-CSF in the present study was much lower than that in previously described studies using a murine MI model in order to mobilize bone marrow stem cells into the peripheral circulation [16,29]. A relatively low dose of G-CSF might be sufficient to promote the wound healing process rather than tissue regeneration [41]. In addition, our results were obtained in an open-chest permanent coronary occlusion model. However, in the practical situation, MI is chiefly caused by spontaneous plaque rupture and thrombus formation, and is mostly treated with reperfusion therapy. A recent clinical trial showed that intracoronary infusion of peripheral blood stem cells mobilized by G-CSF after coronary stenting in MI led to a high rate of restenosis, which was assumed to be due to the effect on neointimal proliferation [52]. The timing and duration of G-CSF administration are also issues that remain to be addressed. TGF-β1 contributes to myocardial hypertrophy and heart failure by acting in concert with angiotensin II [53]. Prolonged upregulation of TGF-β1 might ultimately result in deterioration of the heart’s condition. Further studies including a longer-term observation are needed to confirm the effectiveness and safety of G-CSF therapy.

4.3. Conclusion

Our results indicate that G-CSF treatment improves early post-infarct cardiac remodeling, through, at least in part, promotion of reparative collagen synthesis in the infarcted area, suggesting a beneficial effect of G-CSF on the infarct healing process.

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References


