Evaluation of the Efficacy of Granulocytic Colony-Stimulating Factor for the Treatment of Experimental Myocardial Destruction in Mice

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We studied the effects of recombinant granulocytic CSF on heart remodeling in BALB/c mice after cryodestruction. Administration of granulocytic CSF was started 1 day after cryodestruction (subcutaneously, 10 μg/kg/day, for 4 days). As early as after the first injection, leukocytosis in the peripheral blood started to develop, leukocyte count peaked on days 4-6 and returned to normal on day 14. Treatment with granulocytic CSF significantly increased the content of progenitor cells in the bone marrow and led to rapid development of the inflammatory reaction and myocardium infiltration with mononuclear cells. Injections of granulocytic CSF did not reduce scar area, but provided significantly less pronounced heart hypertrophy, which attests to its better functional properties. By day 30 after cryodestruction, control animals and animals receiving granulocytic CSF exhibited similar morphological picture at the site of damage. Thus, our regimen of granulocytic CSF administration produced a mobilizing effect on bone marrow progenitor cells and postinfarction heart remodeling. Direct effects of granulocytic CSF on the heart have to be established for its use in the treatment of myocardial infarction.

Key Words: *myocardium; postinfarction remodeling of the heart; cell therapy; granulocytic colony-stimulating factor*

Autotransplantation of cell material from the bone marrow is the most preferable variant of using stem cells for stimulation the regeneration processes [1,2]. As an alternative for direct cell transplantation, the possibility for addressed homing of stem cells from the depot to the site of damage is discussed [12]. Cytokines and cytokine-based products are used for this purpose [12]. Granulocytic CSF (G-CSF) is one of the agents affecting bone marrow (BM) stem cells. There are many experimental and clinical reports on the use of G-CSF for correction of postinfarction heart remodeling [5,8,11]. However, no consensus was attained about the cardioprotective properties of G-CSF and this question requires further experimental and clinical studies.

Here we evaluated the effects of recombinant G-CSF on mobilization of multipotent BM stem cell and myocardium regeneration after experimental destruction.

MATERIALS AND METHODS

Experiments were carried out on 30 male BALB/c mice. Myocardium lesion was modeled using cryodestruction technique [9]. To this end, the animal was narcotized, the thorax was opened, and a metal bar cooled in liquid nitrogen (working surface 5 mm^2) was applied without pressing to the myocardium site

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located to the left from the apex for 5 sec. Thereafter the wound was sutured layer-by-layer. Two groups, 15 animals each, were formed. Group 1 mice received recombinant G-CSF (Lenograstim, Aventis) in a dose of 10 μg/kg/day [6] subcutaneously for 4 days starting from the next day after damage. Group 2 animals (control) received the corresponding volume of physiological saline.

Before the experiment and then daily after the intervention, leukocyte count was determined (peripheral blood smears). The animals were killed at the peak of leukocytosis, after normalization of peripheral blood parameters, and 30 days after cryodestruction (5 animals per point). The hearts were washed, weighted, and the area of damage was measured using a micrometer; the samples for histological examination were fixed in 10% neutral formalin, paraffin sections were stained with hematoxylin and eosin.

BM was isolated from the femoral bones of animals killed after normalization of blood parameters and cultured in a medium consisting of 80% DI-MEM, 10% embryonic calf serum, 10% equine serum, 200 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 40 mM hepes-buffer (all reagents from Sigma) at 37°C, 100% humidity, and 5% CO_2. On day 3 of culturing, non-adherent cells were removed during medium replacement [3]. On day 14, the number of colonies and total cellularity were calculated. Cell formations consisting of ≥50 cells were considered as colonies. Similar bone marrow examination was performed in 5 intact animals.

The data were analyzed using ANOVA, Student's *t* test, and Mann–Whitney *U* test.

RESULTS

Peripheral blood examination revealed increased total leukocyte number as soon as 1 day after G-CSF administration. Maximum value (by 2.6 times higher than baseline values) was observed on day 5-6. In the control group, the total leukocyte count increased only 1.3-fold. On day 14, the total leukocyte count in these groups did not differ from baseline values. These findings indicate that G-CSF affected hemopoietic stems in BM. For evaluation of the pool of mesenchymal BM stem cells, BM cells were cultured *in vitro*. After control cryodestruction, the number of colonies was increased almost twofold in comparison with that in control animals (Table 1). The number of colonies in animals receiving G-CSF was even higher. The increase in total cellularity was also noted; particularly in animals receiving G-CSF. Significant increase in the number of colonies after administration of G-CSF suggests that preparation stimulates proliferative processes in BM [6]. The increase in the pool of mesenchymal stem cells in BM can led to their enhanced release into the circulation and increases the probability of migration of progenitor cells into the myocardium. More rapid development of regeneration processes can be expected under these conditions [1,2].

For evaluation of the effects of G-CSF on regeneration capacities of the myocardium, we compared morphometric parameters and histological findings in hearts of animals from group 1 and group 2. Thirty days after cryodestruction, the animals did not differ by the scar area, but significantly differed by heart weight: 140.0±1.3 and 149.2±1.4 mg in groups 1 and 2, respectively. These findings agree with previous reports that G-CSF treatment improved cardiac function after experimental myocardial infarction, but did not reduce scar area [10].

Typical histological preparations of mouse myocardium from groups 1 and 2 on day 6 after cryodestruction are presented (Fig. 1). By this day, morphological structure of the myocardium was similar in the two groups. The formation of a connective tissue scar and minor myocardial degeneration were observed. Major difference between the groups in that period was infiltration of the myocardium with mononuclear cells in animals receiving G-CSF, which was most pronounced in the myocardium adjacent to the damaged area. By day 14, marked cardiomyocyte degeneration was noted (Fig. 2). Cardiomyocyte vacuolization and fine lipid inclusions were noted in animals received G-CSF. The intensity of cell infiltration was several fold higher, than in group 2 and was seen in the myo-

TABLE 1. Effects of Cryodestruction and G-CSF on the Pool of Progenitor Cells in Mouse BM

Parameter	Group		
	intact mice	cryodestruction (control)	cryodestruction+G-CSF
Number of colonies (per flask)	27±7	46±9*	72±12*+
Cellularity, cell/cm^2	2930±253	3520±246*	3885±358*

Note. $p<0.05$ compared to: *intact animals, +control group.

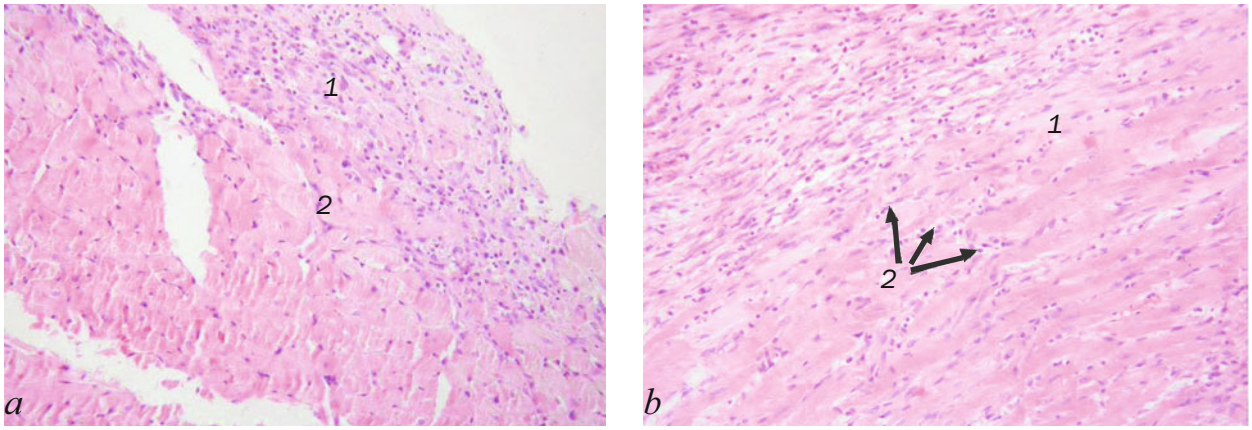

Fig. 1. Morphology of mouse myocardium on day 6 after cryodestruction. Hematoxylin and eosin staining (×200). *a*) control cryodestruction: *1*) formation of connective tissue scar; *2*) slight cardiomyocyte degeneration in adjacent myocardium areas; *b*) animal receiving G-CSF: *1*) slight cardiomyocyte degeneration; *2*) mononuclear infiltration in adjacent myocardium areas.

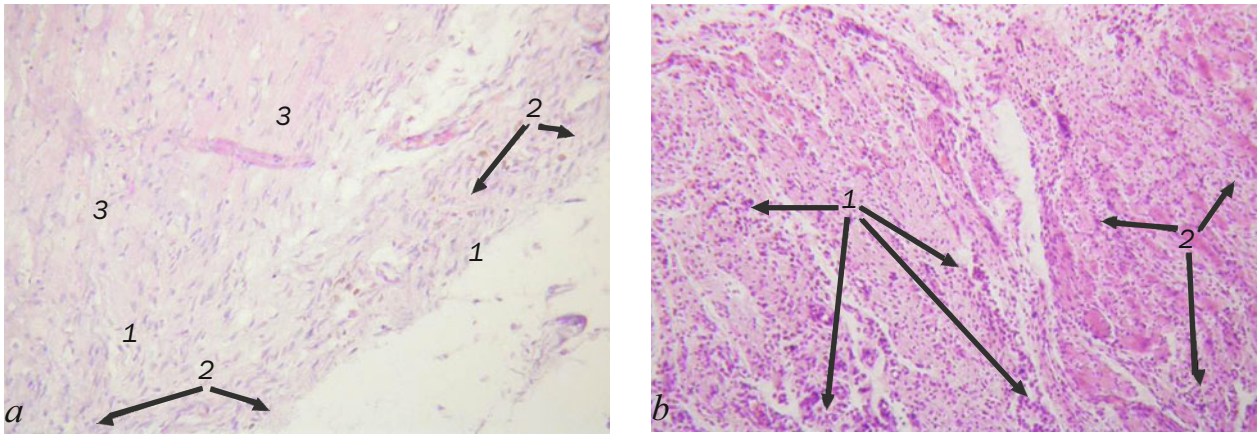

Fig. 2. Morphology of mouse myocardium on day 14 after cryodestruction. Hematoxylin and eosin staining. *a*) control cryodestruction (×200): *1*) formation of loose connective tissue scar, *2*) mild mononuclear infiltration in the zone of cryodestruction, *3*) cardiomyocyte dystrophy in adjacent areas; *b*) animals receiving G-CSF (×100): *1*) marked infiltration with lymphocytes, monocytes, and neutrophils in the zone of cryodestruction, *2*) cellular infiltration in myocardium adjacent to the zone of lesion.

cardium adjacent to the site of cryodestruction and in more remote areas. In group 2, cell infiltration was less pronounced than on day 6 and was observed only in the area of cryodestruction. In animals receiving G-CSF, the connective tissue was more compact than in the control group, where it has loose structure. By day 30, morphological structure in two groups was virtually identical (Fig. 3). Fibrous connective tissue was formed at the site of destruction and sites of vacuolated cardiomyocytes and hemorrhages remained in the adjacent areas.

These findings indicates that G-CSF accelerated organization in the damaged zone, probably due to higher content of progenitor cells. Thus, G-CSF induced a transient increase in the content of colony-stimulating cells in the myocardium [4]. However, there is no agreement concerning the nature of these cells. Some authors believe that it is a result of direct effect of G-CSF on the myocardium and regional stem cells [7,8].

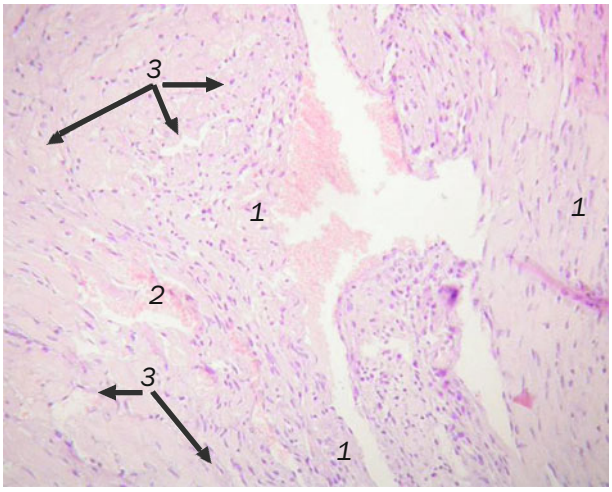

Fig. 3. Typical morphological picture of the myocardium in animals from the control and experimental groups on day 30 after cryodestruction. Hematoxylin and eosin staining (×200). *1*) connective tissue scar, *2*) hemorrhage, *3*) vacuolation of cardiomyocyte cytoplasm in areas adjacent to the scar.

Thus, we can say that G-CSF administered by the specified scheme affects BM progenitor cells. Stimulation of reparation in the myocardium after application of G-CSF is determined by activation of processes accelerating connective tissue formation. The use of G-CSF must be considered as a component of complex therapy of myocardium infarction. For higher efficacy of G-CSF, its direct effects on myocardium cells should be more comprehensively studied.

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