PHARMACOLOGY AND TOXICOLOGY

Effect of Granulocyte Colony-Stimulating Factor on Myocardium Recovery in Postinfarction Period

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The effect of Neutrostim (preparation of granulocytic colony-stimulating factor) on recovery of myocardial tissue after acute myocardial infarction was studied in rats. A course of Neutrostim after ligation of the left coronary artery led to normalization of electrocardiographic and morphological parameters of the myocardium after one month.

Key Words: *myocardial infarction; regeneration; stem cells*

Coronary heart disease ranks first among cardiovascular diseases in adults. Coronary disease and its acute form (myocardial infarction) often lead to the development of heart failure (HF) [1]. Modern methods for the prevention and treatment of HF employing a wide spectrum of drugs and non-drug approaches remain low effective, because they do not arrest the progressive course of HF development and do not reduce its incidence [6]. None of the methods used in practical cardiology eliminates the main cause of HF: replacement of myocardial tissue with non-contracting connective tissue [4].

Great attention is paid to cell therapy of HF as a method aimed at repair of the functional activity of the myocardium [5,8,9]. For example, methods for mobilization of patients own bone marrow mesenchymal stem cells (MSC) from depot by injection of specific mobilizating factors, *e.g.* granulocytic colony-stimulating factor (G-CSF) and stem cell factor (SCF) are now in progress. Preventive combined injection of these factors to mice with experimental myocardial infarction gave positive results [7].

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Here we studied the effect of G-CSF administered to rats with experimental acute infarction on myocardial recovery during the postinfarction period.

MATERIALS AND METHODS

Experiments were carried out on 36 male Wistar rats (250-300 g). The animals were kept in accordance with the regulations of the European Convention on Protection of Vertebrates Used for Experimental and Other Research Purposes.

After thoracotomy (ether narcosis) myocardial infarction was modeled by ligation of the left coronary artery at the level of the first quarter of the distance from the pulmonary cone to the heart apex under with visual and electrocardiographic (ECG) monitoring. After the intervention the rats with ECG signs of heart ischemia were divided into 2 equal groups. Experimental animals were injected with a G-CSF preparation Neutrostim in a dose of 100 mg/kg in 0.5 ml saline subcutaneously daily for 5 days (the preparation was developed at Vektor Company in cooperation with Institute of Pharmacology, Tomsk Research Center). The first dose was injected 3 h after surgery. Controls were injected with 0.5 ml saline according to the same protocol.

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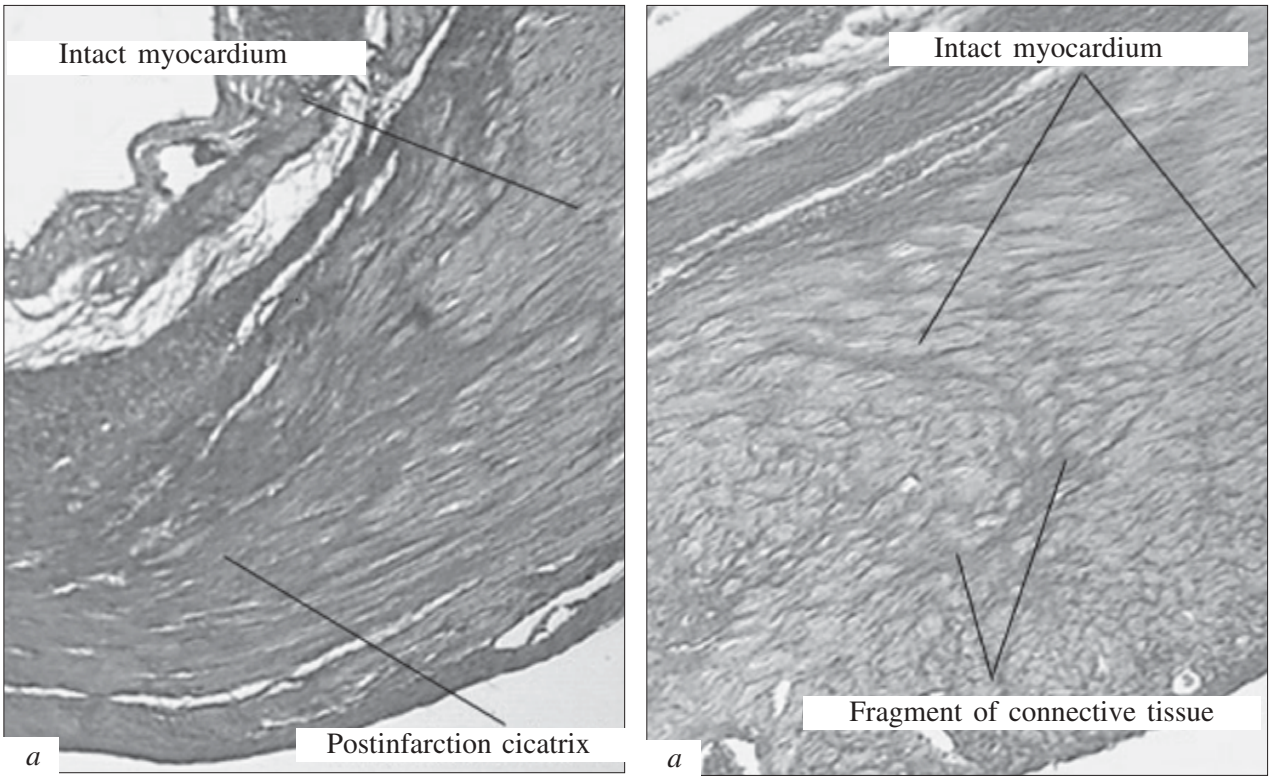

Fig. 1. Myocardium of rats with experimental myocardial infarction on day 30 after coronary occlusion. *a*) animals receiving saline; *b*) Neutrostim treatment; ×80.

ECG studies were carried out in all animals before, 3 h and 30 days after coronary artery ligation. On day 30 of the experiment the animals of both groups were sacrificed by ether overdose, the thorax was opened, and the heart was removed.

For morphological study the aorta and right ventricle (without ventricular septum) were placed into 10% formalin. After routine dehydration the left ventricle was embedded into paraffin. Sections (5 μ) were made through the entire left ventricle from the base to the apex every 300 μ. The sections were stained with picrofuchsine for connective tissue and photographed (the entire section area) using a Digital micro microvideocamera (Elecart). The ratio of myocardial structural elements was evaluated on microphotographs using computer software for graphic data processing.

The data were processed by methods of variation statistics using Statistica 5.0 software.

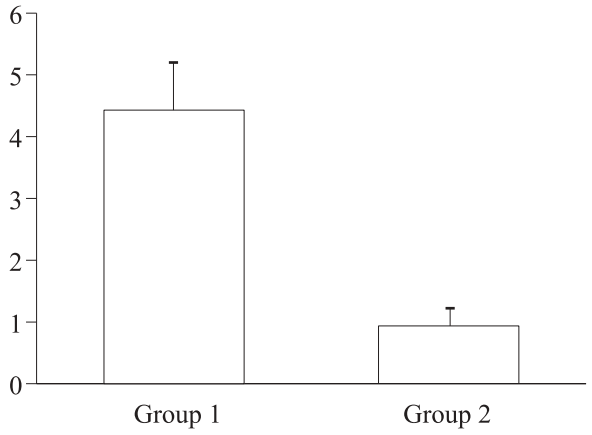

Fig. 2. Percentage of connective tissue in the myocardium of rats with experimental myocardial infarction on day 30 after coronary artery ligature. *1*) animals receiving saline; *2*) Neutrostim treatment. Ordinate: percentage of connective tissue. Confidence intervals at p=0.05.

RESULTS

Animal mortality during the early period (day 2) after coronary artery occlusion was 34%. Later all animals treated with Neutrostim survived, while 6% controls died, presumably, from progressive HF.

The initial ECG values in experimental and control groups were virtually the same and were normal for these animals [2]. Pronounced ECG changes developed 3 h after coronary occlusion (Table 1). The amplitude of *T* wave sharply increased, indicating the development of significant ischemic disorders in the myocardium. This was paralleled by a decrease in *R* wave amplitude and by the appearance of pathological *Q* wave (in 42% animals). *QRS* complex was absent

TABLE 1. Heart Rate and ECG Parameters in Rats with Experimental Myocardial Infarction ($X \pm m$)

Parameter	Control			Experiment		
	initial values	3 h	30 days	initial values	3 h	30 days
Heart rats, min^{-1}	417±10	437±10	347±13^{+}	413±10	443±18	398±16*
PQ, msec	50±1	47±3	50±2	48±1	47±2	47±2
QT, msec	78±3	81±2	94±5^{+}	79±4	80±4	79±4*
QRS, msec	15±1	14±2	18±2	14±1	12±1	17±2
P, μV	32±4	22±5	26±5	29±4	30±5	27±5
R, μV	272±22	154±30^{+}	149±20^{+}	293±16	206±24^{+}	328±44*
T, μV	92±21	220±41^{+}	93±9	113±16	208±31^{+}	140±26
Q, μV		87±41^{+}	5±1^{+}		77±24^{+}	

Note. $p<0.05$ compared to *control, $^{+}$initial value.

in some cases and *QT* complex was recorded. Changes in *QRS* complex reflect the formation of an extensive necrotic zone in the myocardium. Shifts in ECG parameters indicated the development of acute stage of myocardial infarction [3].

Thirty days after coronary occlusion *T* wave in experimental rats virtually returned to normal, which attested to disappearance of the ischemic damage zone. In controls the amplitude of *R* wave remained low (140±26 μV). Heart rate was also low (347± 13 min^{-1}) and ventricular "electric" systole was decelerated (prolonged *QT* interval). Pathological *Q* wave was detected in one animal. These ECG changes indicate postinfarction cardiosclerosis (cicatricial stage of myocardial infarction) in control rats.

The amplitude of *R* wave in animals treated with Neutrostim was 328±44 μV on day 30 after coronary occlusion, which was significantly higher than in the control group. Heart rate was 13% higher and *QT* interval was significantly shorter compared to the control. ECG of these rats improved not only in comparison with the control; moreover, there were no statistically significant differences in comparison with the ECG values before myocardial infarction modeling.

Hence, the course of Neutrostim therapy in rats with ligated coronary artery normalized ECG parameters by day 30.

Analysis of histological preparations showed a connective tissue cicatrix at the site of necrotic zone in experimental animals 30 days after infarction. In the majority of controls the heart wall in the cicatrix zone consisted of connective tissue (Fig. 1, *a*). The percentage of connective tissue elements in the myocardium on the preparations was 4.43±0.93. In animals treated with Neutrostim postinfarction sclerosis was less pronounced: collagen fibers in the cicatrix zone were alternating with cardiomyocytes, occupying just 0.39±0.15% of total myocardial area (Fig. 1, *b*; Fig. 2).

Presumably, the course of Neutrostim mobilizing the bone marrow MSC [7] effectively stimulated regeneratory processes in the myocardial tissue after ischemic damage. After mobilization and homing the precursor cells differentiated into working myocardial tissue, thus preventing the development of connective tissue cicatrix and increasing functional activity of the myocardium during the postinfarction period.

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