Transplantation of Bone Marrow Mononuclear Cells Does Not Affect Postinfarction Electrical Remodeling of the Heart

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We studied the effect of allotransplantation of bone marrow mononuclear cells on postinfarction remodeling of the heart in rats. The cells were transplanted into the periinfarction zone of the heart. The transplantation was performed on day 9 after coronary occlusion. It was found that on day 45 after coronary occlusion myocardial hypertrophy developed, ventricular fibrillation threshold decreased, but myocardial contractility remained within the normal. Allotransplantation of bone marrow mononuclear cells had no effect on myocardial hypertrophy and did not prevent the development of electrical instability of the heart.

Key Words: *stem cells; postinfarction remodeling of the heart; regeneration*

Postinfarction remodeling of the heart is an actual problem of modern cardiology, because this pathological process eventuates in heart failure or electrical instability of the heart, which, in turn, is the main cause of sudden cardiac death in patients with postinfarction cardiosclerosis [14]. Inhibitors of angiotensin-converting enzyme and β-adrenoblockers are the most effective preparations for prevention of this pathology [2]. The therapeutic potential of these drugs is limited, and therefore the search for new approaches to prevention of postinfarction remodeling of the heart and stimulation of heart regeneration and neovasculogenesis after myocardial infarction is now in progress [1]. Studies of stem cells (SC), cardiomyocyte and endotheliocyte precursor cells, are very important in this respect [1]. These cells can be transported with the blood into the myocardium and differentiate into endotheliocytes, cardiomyocytes, or vascular smooth muscle cells. These data provided the basis for the experiments on cell therapy of myocardial infarction [6,8,15]. However, the question which cells and when should be transplanted into the myocardium is still open, delayed positive and negative consequences of cell therapy are unknown. The most comprehensive clinical experience was gained in studies of transplantation of heterogeneous fraction of bone marrow mononuclear cells (BMMC) [6,8,15]. There is still no consensus on the efficiency of this cell therapy. For instance, some authorities observed improvement of the pumping function of the heart [6,8,15] and coronary perfusion [6,15] after transplantation of BMMC, while others reported that cell therapy was unable to prevent postinfarction remodeling of the heart [4,7] and produced only minor (by 7% compared to the initial value) increase of ejection fraction [4] or did nor prevent the development of heart failure at all [7]. The effect of BMMC transplantation on electrical instability of the heart resulting from postinfarction cardiofibrosis remains practically unstudied.

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Here we studied the effect of BMMC transplantation on the process of postinfarction remodeling and electrical stability of infracted myocardium.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 250 g. Some animals were used for modeling experimental myocardial infarction, others served as the source of BMMC. Coronary occlusion was performed under chloralose narcosis (50 mg/kg intraperitoneally) under conditions of jet ventilation. BMMC were isolated by gradient ultracentrifugation [9]. In this case, the mice were decapitated under ether narcosis. For obtaining bone marrow suspension, mouse femurs were perfused with 5 ml DMEM (No. D 3656 "Sigma") containing BSA fraction V (1%) and heparin (100 U/ml) through a syringe. The material was resuspended through needles of decreasing diameters for obtaining homogeneous suspension that was then layered onto 3 ml Ficoll-hypaque (1.077 g/cm^3, Pharmacia) and centrifuged at 1500 rpm for 20-30 min. The interphase ring was collected and washed twice from Ficoll-hypaque by centrifugation at 1500 rpm for 20-30 min. After the last centrifugation the cells were resuspended in 1 ml DMEM. The total number of isolated mononuclears was counted in a Goryaev chamber. Cell viability was evaluated by exclusion of 0.1% trypan blue [9]. For cytological analysis, an aliquot of cell suspension was transferred onto slides, dried, fixed for 5 min in methanol, and stained with azure II and eosin. BMMC suspension was adjusted to a concentration of 10^7 viable cells per 1 ml medium with DMEM.

Allotransplantation was performed on day 9 after coronary occlusion. The term of cell transplantation was chosen on the basis of published data [4]. The chest was opened and BMMC (4×10^6 mononuclear cells per rat) were injected into the wall of the left ventricle to a depth of 0.5-1.0 mm along the periphery of the necrotic zone. The operation wound was sutured layer-by-layer. The control animals with coronary occlusion received DMEM via the same route. The ventricular fibrillation threshold (VFT) was measured after 45 days, when the formation of postinfarction scar was completed. Myocardial hypertrophy was evaluated by the ventricular weight/body weight ratio. Dilatation of the left ventricle was evaluated by its volume.

Myocardial hypertrophy can be compensated or decompensated. In the later case, the pumping function of the heart decreased, which leads to heart failure. To determine the degree of myocardial hypertrophy (compensated or decompensated), a series of experiments was carried out on isolated perfused hearts obtained from intact rats and animals with postinfarction cardiofibrosis (in this case, cell transplantation was not carried out). After thoracotomy under ether narcosis the heart was promptly removed and arrested in cold (4°C) Krebs—Henseleit solution. Then, retrograde Langendorf perfusion of the heart was started. For evaluation of the contractile function, the heart was perfused with carbogen-saturated Krebs—Henseleit solution (37°C, pH 7.4) containing (in mM): 120 NaCl, 4.8 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20.0 $NaHCO_3$, 10.0 glucose under conditions of constant pressure 52 mm Hg. After 30-min stabilization period, HR and contractility parameters of the isolated heart were recorded: left-ventricular developed pressure (LVDP), end-diastolic pressure (EDP, % of initial values). Contractile force was evaluated by LVDP calculated as the difference between systolic and diastolic presusure. Additionally, contraction and relaxation rates were calculated.

The data were processed using Statistica 6.0 software (StatSoft Inc.). The differences were significant at $p<0.05$.

RESULTS

Myocardial hypertrophy developed 1.5 month after coronary occlusion (Fig. 1). In rats with cardiofibrosis, the ratio of ventricular weight to body weight increased by on average 28% compared to intact animals. Hypertrophy was concentric, because dilatation of the left ventricle was not observed. Electrical instability of the heart developed simultaneously with hypertrophy: in control animals with cardiosclerosis, VFT was 4-fold lower

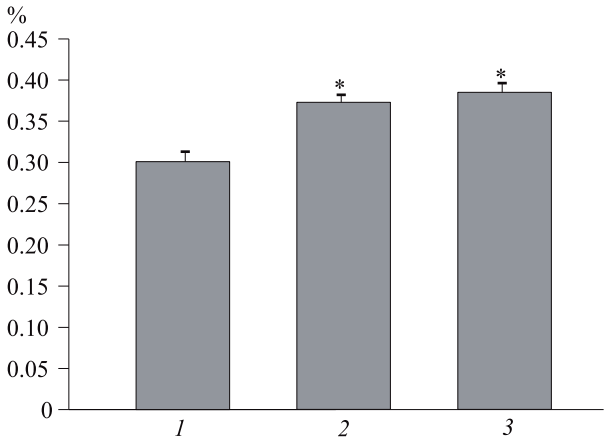

Fig. 1. Ventricular weight/body weight ratio in rats with cardiofibrosis. Here and on Figs. 2: *1*) intact animals, *2*) BMMC transplantation, *3*) control. *$p<0.01$ compared to intact animals.

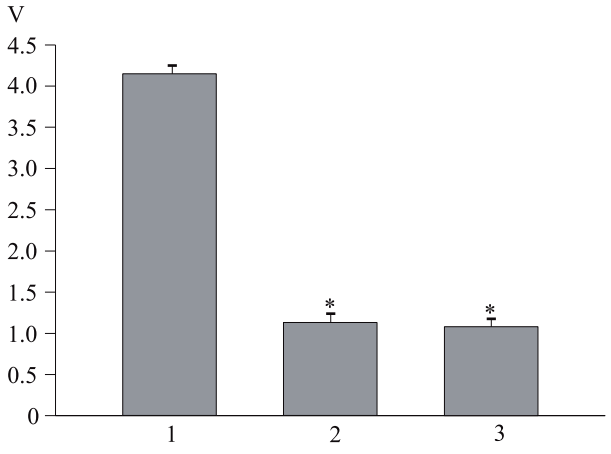

Fig. 2. Ventricular fibrillation threshold in animals with cardiosclerosis.

than in intact rats (Fig. 2). The correlation coefficient (r) between the weight of the left ventricle and VFT was 0.5 (n=36). Allotransplantation of mononuclear cells did not affect the pattern of myocardial hypertrophy and the volume of the left ventricle (Fig. 1). VFT in rats receiving cell therapy did not differ from the analogous parameter in rats receiving DMEM.

HR in animals with postinfarction cardiosclerosis was 183±20 (n=14) *vs.* 198±22 bpm (n=14) in intact animals. These differences were insignificant. LVDP in intact rats was 58.8±5.6 mm Hg (n=14) and in rats with myocardial infarction 48.5±5.9 mm Hg (n=14). The maximum contraction rate in the control was 38.1±9.3 mm Hg/sec (n=14) and for heart with cardiosclerosis 30.8±5.6 mm Hg/sec (n=14). The maximum relaxation rates were 25.5±6.4 (n=14) and 21.9±4.5 mm Hg/sec (n=14), respectively. No significant differences between the groups by EDP were also revealed.

Our findings confirmed the development of heart hypertrophy within 45 days after coronary occlusion. Parameters of contractility for sclerosed heart were lower than for intact, but the differences between the control and experimental groups were insignificant. This fact attests to compensatory nature of heart hypertrophy after myocardial infarction not accompanied by the development of heart failure.

In animals with postinfarction scar, a 4-fold decrease in VFT was observed. VFT did not correlate with ventricular weight, which also is an indirect sign of compensatory type of hypertrophy. Indeed, decompensated hypertrophy usually leads to electrical instability of the heart; therefore, a correlation exists between the size of the heart and its electrical instability [5]. In our case, no relationships of this kind were noted, which attests to compensated type of hypertrophy in rats with postinfarction cardiosclerosis.

Allotransplantation of BMMC had no effect on the pattern of postinfarction hypertrophy. This can be explained by 1) death of transplanted cells due to their rejection and 2) compensated type of hypertrophy, *i.e.* the decease in the weight of the left ventricle can led heart failure. It is known that after intramyocardial allotransplantation BMMC persist in the myocardium for at least 14 days [3]. Neonatal cardiomyocytes after transplantation into the myocardium of adult animals survive for 6 months [13]. In light of this, we hypothesized that the absence of the effect of cell cardiomyoplasty on the weight of the heart ventricles is related to compensated type of myocardial hypertrophy in experimental animals and to the fact that the decrease in myocardial weight in this case is biologically inappropriate.

The effect of cell cardiomyoplasty on electrical stability of the myocardium was discussed previously [10]. There are experimental data that intracardiac transplantation of autologous myoblasts from skeletal muscles in many patients provokes ventricular tachycardia resistant to antiarrhythmic drugs [12]. It was interesting to evaluate the effect of intramyocardial injection of BMMC on electrical stability of the heart. Our experiments showed that allotransplantation of BMMC had no effect on VFT.

Thus, allotransplantation of BMMC had no effect on postinfarction remodeling of the heart. Cell cardiomyoplasty did not modulate electrical instability of the heart in postinfarction cardiosclerosis.

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