CELL TECHNOLOGIES IN BIOLOGY AND MEDICINE

Effect of Stress-Proteins on Survival of Bone Marrow Mesenchymal Stem Cells after Intramyocardial Transplantation against the Background of Postinfarction Heart Remodeling

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We studied the presence of colony-forming cells in cell culture from rat heart 40 days after experimental myocardial infraction. The mean cellularity in this pathology was 12±8 cell/cm^2, which is 20-fold lower than in intact myocardium. Transplantation of mesenchymal stem cells into the remodeling myocardium restored the pool of colony-forming cells. This effect depended on the state of transplanted cells. After transplantation of mesenchymal stem cells with low content of stress proteins, 6±2 colonies were detected, while after transplantation of cells with high content of hsp70 and hsp60 stress proteins (modified mesenchymal stem cells) 18±5 colonies were found, the mean cellularity of the corresponding cultured being 946±267 and 1926±123 cell/cm^2. The positive effect of modified mesenchymal stem cells was observed on days 4 and 7 after transplantation. We conclude that postinfarction remodeling mobilized the total pool of regional stem cells; mesenchymal stem cells with high content of hsp70 and hsp60 demonstrated highest survival rate after intramyocardial transplantation.

Key Words: *myocardium; postinfarction remodeling; cell transplantation; mesenchymal stem cells; stress proteins*

Isolation of cell material on the basis of bone marrow stem cells (SC) for further transplantation for the maintenance and restoration of functional activity of damaged organs and tissues is an important trend in cell technologies [3]. Intracoronary or intramyocardial transplantation of SC was proposed for the treatment of heart failure of different genesis [1]. In the latter case, the transplanted SC from optimal environment maintained during culturing are transferred into pathologically damaged myocardium. Among unfavorable factors negatively affecting transplanted cells the most important role is played by expanded and dense extracellular matrix, oxygen deficiency, and the procedure of transplantation. The number of viable cells cannot be increased by increasing the dose of transplanted cells, because this leads to activation of apoptosis processes. However, there are endogenous mechanisms of adaptation at the cellular level, which can considerably improve cell resistance to unfavorable

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factors. These mechanisms can be stimulated by short-term exposure to stress factors, *e.g.* increased temperature. Synthesis of heat shock proteins is a universal mechanism improving structural and functional resistance of cells in various pathological states. It can be hypothesized that similar effect will be observed in individual SC in response to stimulation of the synthesis of inducible stress proteins at the stage of culturing of these cells. The possibility of initiation of the synthesis of stress proteins at the level of cell culture was previously demonstrated [5,6]. It is possible that these modified cells better endure the procedure of intramyocardial transplantation and form mesenchymal islet colonies in the myocardium. It can be expected that the formation of these colonies and their functioning will provide proliferative activity of SC and natural increase in their number.

Here we evaluated survival of bone marrow mesenchymal SC (MSC) with initiated synthesis of inducible stress proteins after intramyocardial transplantation into pathologically changed myocardium.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 180-230 g. Damage to the myocardium was induced by coronary occlusion [4]. The thorax and the pericardium were opened under light ether anesthesia and a ligature was applied on the upper third of the left descending coronary artery. The wound was treated with antibiotic and sutured layer-by-layer after air discharge from the thorax. According to published reports and our data (Fig. 1), dead cardiomyocytes are replaced with the connective tissue and a scar is formed in the zone of necrosis 40 days after this procedure; heart hypertrophy develops [4].

The animals were divided into 3 groups (6 rats per group). The thorax was opened again and 100 μl culture medium was injected intramyocardially. In groups 2 and 3, the medium containing 1-2×10^5 MSC and modified MSC (MMSC) was injected, respectively. Group 1 animals served as the control: they received medium containing no cell material. The medium was injected into the myocardium adjacent to the scar (5-6 points). After the second surgery, the animals were maintained under vivarium conditions for 4 days.

For isolation of MSC, the mononuclear fraction of the bone marrow from the femur of 4 intact animals was used. The bone marrow was homogenized by squeezing through a Capron mesh with pore diameter of 100 μ. The cell mass was centrifuged at 2000 rpm for 20 min. The cell suspension

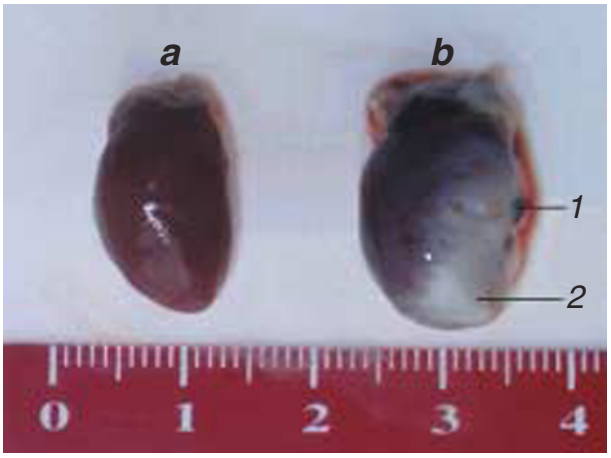

Fig. 1. Intact and remodeled rat hearts. *a*) heart of intact animal; *b*) heart on day 40 after coronary occlusion. 1) site of artery ligation; 2) scar zone.

was layered on Ficoll-Hypaque (1.077 g/cm^3 density gradient). The cell ring on the gradient was collected and washed with RPMI-1640 medium containing BSA. Viability of mononuclears and total cellularity of the material were evaluated [7]. The number of viable cells was adjusted to 5×10^6 cell/ml, transferred to 50-ml plastic flasks (10 ml per flask), and cultured for 14 days [8]. The medium was then discharged and the feeder layer was treated with 0.1% trypsin and collagenase. The cell suspension was transferred into tubes and washed from the enzymes with RPMI-1640 medium. The number of viable cells and total cellularity were determined.

It is known that heat stress is a universal factor initiating synthesis of stress proteins in cells, including cultured cells [5,6]. Dosed heat exposure was used for preparing MMSC. To this end, culture flasks with cells were successively placed into thermostats with different temperature regimens 1 day before the end of culturing: 15 min at 43°C, 4 h at 37°C, 2 h at 43°C, and 20 h at 37°C.

The effect of the modifying procedure was evaluated by measuring the content of stress proteins in cells at the end of culturing using commercial ELISA Kit for hsp70 and hsp60 stress proteins.

Four days after transplantation, the experimental and 6 intact rats were sacrificed, the hearts were isolated under sterile conditions, washed with sterile physiological saline, and the wall of the left ventricle was isolated. The myocardium was minced and treated with 0.25% typsin-EDTA and culture flasks incubated at 37°C for 10 min [9]. Further desaggregation of the tissue was performed by passing it through needles with decreasing diameters and by filtering through a Capron mesh (pore diameter 100 μ). The cell suspension was twice centrifuged in RPMI-1640 with BSA. The pellet was

TABLE 1. Results of Culturing of Rat Myocardial Cells from the Left Ventricle (*M*±*m*)

Parameter	Intact (*n*=6)	Experimental groups			
		group 1 (*n*=6)	group 2 (*n*=6)	group 3 (*n*=6)	
				day 4 (*n*=6)	day 7 (*n*=6)
Number of colonies	0	0	6±2*	18±5*	29±9*
Cellularity, cell/cm^2	246±60	12±8*	946±267*	1926±123*	1787±396*

Note. Group 1: animals with postinfarction cardiosclerosis (PICS) and injection of culture medium; group 2: animals with PICS and injection of MSC; group 3: animals with PICS and injection of MMSC. *p<0.01 compared to intact myocardium.

resuspended in 4 ml culture medium. The total cellularity and viability of nuclears were evaluated. The optimal volume of the cell suspension for seeding into 50-ml culture flasks was determined. The volume of cell suspension was brought to 5 ml with culture medium and incubated routinely for 16 days [4]. The medium was then removed, the flasks were dried, and their content was fixed and stained. The number of colonies and total cellularity of the preparations were determined [7,9]. The data were processed by methods of variation statistics using Student *t* test.

RESULTS

The results of culturing of myocardial cells differed considerably between the groups (Fig. 2). After culturing of myocardial cells from intact animals, none cell colonies were found, the total cellularity being 246±60 cell/cm^2 (Table 1). Culturing of the myocardial cells from animals with modeled postinfarction cardiosclerosis also yielded no colonies, but cellularity of the culture was lower by 20 times (12±8 cell/cm^2). Other results were obtained in animals with modeled heart pathology and single intramyocardial transplantation of MSC. Four days after transplantation, the culture of myocardial cells from these animals (Table 1) contained 6±2 colonies, while cellularity of these samples was 946±267 cell/cm^2, which was almost 4-fold higher than in intact myocardium. Taking into account relatively short period from cell transplantation to the start of culturing of myocardial cells and small pool of intrinsic SC in the pathological myocardium, we can conclude that this result is most likely determined by replenishment of the cell pool at the expense of the transplant. Transplantation of MMSC considerably affected the results of culturing. In this case, the mean cellularity increased by more than 8 times compared to intact myocardium; 18±5 colonies were found (Table 1).

The data obtained during culturing suggest that single transplantation of MMSC ensured greater pool of colony-forming cells. This can be explained by higher resistance of MMSC to unfavorable factors acting on cells during transplantation. It is known that thermal exposure applied in our experiments can increase cell resistance to unfavorable factors due to initiation of the synthesis of inducible stress proteins [5,6]. Analysis of MSC and MMSC used for transplantation revealed principal differences between these cells by the content of these proteins (Table 2). The concentrations of stress proteins hsp70 and hsp60 in 1 ml culture medium containing 10^7 cells were 0.18±0.02 and 1.36±0.04 μg/ml, respectively. In MMSC, the concentration of hsp70 increased by 85 times and the concentration of hsp60 increased 2 fold (to 15.28±0.09 and 2.73±0.05 μg/ml, respectively.

The scheme for obtaining MSC from the bone marrow provides optimal conditions for cells and creates the possibilities for additional initiation of the synthesis of inducible stress proteins hsp70 and hsp60 (Table 2). However, the observed differences in induction of hsp70 and hsp60 in response to heat exposure reflect relative isolation of mitochondria as intracellular compartments carrying their own DNA [12,14].

Predominant increase in the content of hsp70 in MSC at the stage of transplantation is most favorable, because these proteins are located in the premembrane layer of the cytoplasm and interact with macromolecular structures [10]. They can also interact with proteins of the cytoskeleton, though greater part in these processes is played by stress proteins with lower molecular weight [11]. Antiapoptotic effect of hsp70 [13] was also demonstrated. This can be most significant at the stage of

TABLE 2. Results of ELISA of Stress Proteins in Cell Culture (*M*±*m*)

Parameter	MSC (*n*=6)	MMSC (*n*=6)
hsp70, μg/ml	0.18±0.02	15.28±0.09*
hsp70, μg/ml	1.36±0.04	2.73±0.05*

Note. *p<0.05 compared to MSC.

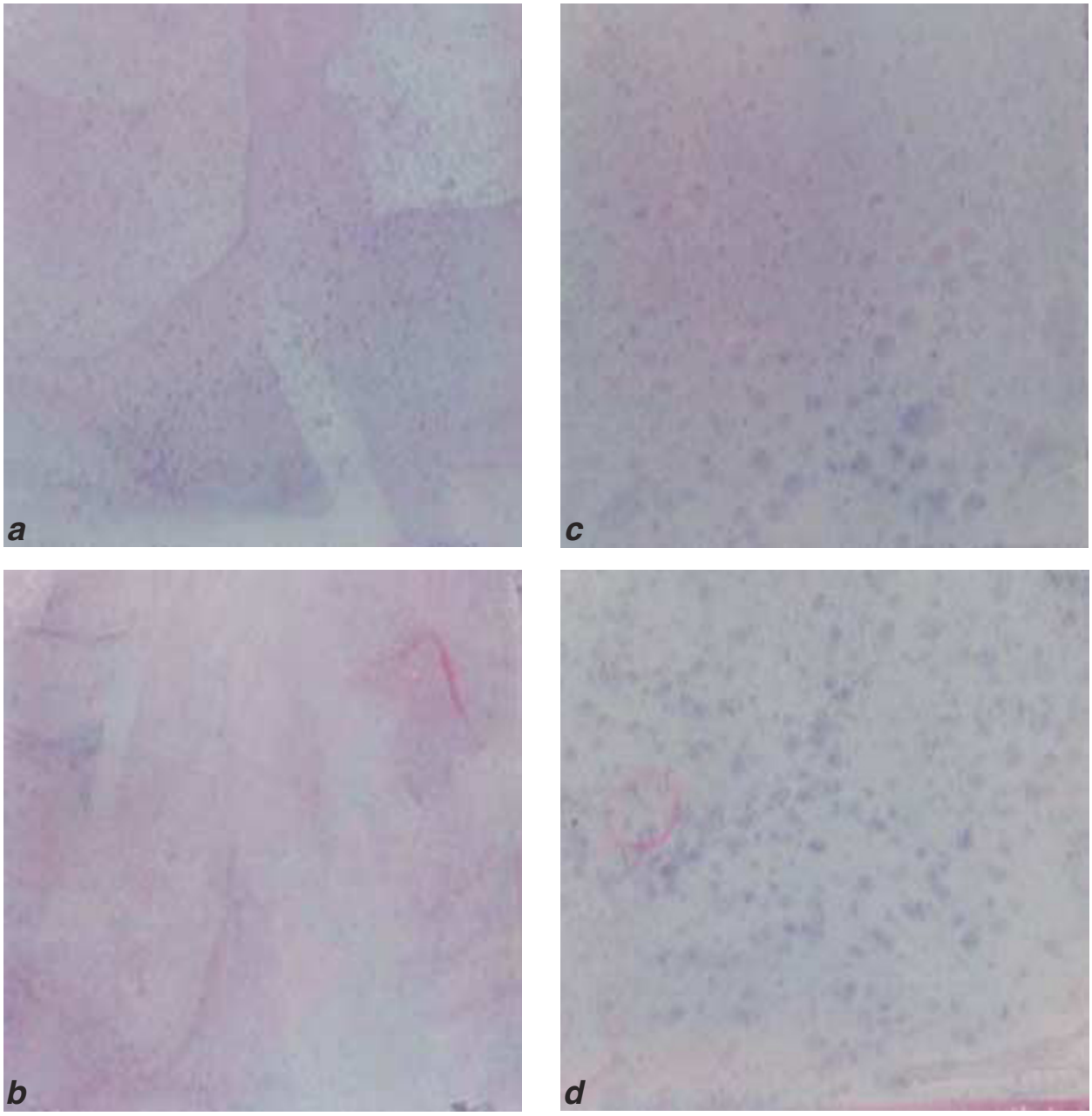

Fig. 2. Surface of flasks after 16-day culturing of myocardial cells. *a*) intact myocardium; *b*) group 1 (remodeled myocardium after coronary occlusion); *c*) group 2 (day 4 after injection of MSC into remodeled myocardium); *d*) group 3 (day 4 after injection of modified MSC into remodeled myocardium).

cell distribution immediately after transplantation. At the same time, it was hypothesized that the protective effect of stress proteins is not very important under conditions of chronic pathology or long-term exposure to unfavorable factors [10]. That is why viable cells can die at later terms after transplantation under the effect of unfavorable microenvironment. For verification of this hypothesis, culturing of myocardial cells obtained from 6 animals was started on day 7 after MMSC transplantation (instead of day 4). In this case, the pool of colony-forming cells in the myocardium was retained compared to this parameter on day 4 and even a tendency towards an increase in the number of formed colonies was noted (Table 1). This result suggests that the procedure of transplantation is most crucial for cell survival after their intramyocardial transplantation into pathologically changed myocardium. The cytoprotective effect of hsp70 and hsp60 was sufficient for effective intramyocardial transplantation of cells.

Thus, the endogenous pool of resident colony-forming cells in the myocardium is exhausted during postinfarction remodeling. On day 40 after ischemic damage, natural homing of SC from the bone marrow did not restore the pool of resident colony-forming cells. The standard scheme of MSC culturing creates conditions for additional activation of the synthesis of stress proteins in these cells. MSC with high content of hsp70 and hsp60 are characterized by high survival rate after transplantation into remodeled myocardium. This modification of MSC at the stage of their culturing can increase the efficiency of cell transplantation in the therapy of cardiovascular diseases.

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