Effect of Cell Transplantation on Induction of Heat Shock Proteins in Damaged Heart

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Variations in the amount of heat shock proteins of various classes in the damaged myocardium of rats were studied after xenogeneic transplantation of heart cells.

Key Words: *heat shock proteins; neonatal cells; epinephrine-induced heart injury*

Heat shock proteins (Hsp), or stress proteins, play an important role in the system of intercellular communication. Previous studies showed that these proteins are involved in cell protection during the formation of stress-induced regulatory cascades [9]. The involvement in cell processes indicates that heat shock proteins play the major role in the reparation and degradation. Dysfunction of Hsp contributes to functional disturbances and damage to organs and tissues [8]. Some authors believe that heat shock proteins constitute a intracellular stress-limiting system, which protects the cell from damage [2,3,12].

A large body of evidence indicates that cell transplantation improves the course of some diseases. Therapeutic effect of the transplant cannot be explained only by replacement of some abnormal cells [6,10]. Little is known about the intracellular mechanisms of organ protection. These mechanisms are induced by transplanted cells. Evaluation of the role of heat shock proteins in cell damage and the effect of cell transplantation on this process is of scientific and practical importance.

Here we studied the role of heat shock proteins in cell damage. The effect of cell transplantation on this process was evaluated.

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MATERIALS AND METHODS

The effect of neonatal cell suspensions was studied on male outbred albino rats (n=144) weighing 180-210 g. The study was performed in the fall-winter period. The age of animals was above 6 months. The suspension of heart cells from newborn rabbits (1-2 days) was obtained at the Laboratory of Biologically Active Substances (Center for Reconstructive and Repair Surgery, East-Siberian Research Center, Siberian Division of the Russian Academy of Medical Sciences). This suspension was transplanted to experimental animals. The method for preparation and isolation of neonatal cells was described previously [7].

The animals were divided into 3 groups. Epinephrine-induced heart injury in group 1 rats (n=56) was modeled by the method of L. M. Nepomnyashchikh (1981). These animals received a subcutaneous injection of epinephrine (5 mg/kg into the right thigh) and subcutaneous injection of physiological saline (0.5 ml). Neonatal cells were transplanted to group 2 rats (n=64). The suspension of neonatal cells from rabbit heart (500,000 cells in 0.5 ml physiological saline) was injected subcutaneously into the left thigh immediately after treatment with epinephrine. Healthy rats of the control group (n=24, no injection of epinephrine) received subcutaneously the suspension of rabbit heart cells in an equivalent dose and volume.

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The myocardial tissue and blood serum were sampled 1, 4, 8, 12, and 16 h and 1, 3, and 7 days after the start of experiments.

The concentration of Hsp-70, Hsp-72, and Hsp-60 and the amount of small heat shock proteins sHsp-32 and sHsp-27 were measured in the heart tissue of experimental animals. The isolation of water-soluble protein from the myocardium and subsequent electrophoresis were performed by the method of Laemmli on a Mini-PROTEIN II electrophoresis device (Bio-Rad). Constitutive and inducible isoforms of heat shock proteins were identified with primary antibodies against Hsp-70, Hsp-72, Hsp-60, sHsp-32, and sHsp-27 (Sigma and StressGen). Specific amino acid sequence of primary antibodies for a certain family of heat shock proteins was detected using secondary antibodies (Sigma) conjugated with alkaline phosphatase. The membranes were scanned and analyzed by Sigma Scan Pro software. The relative content of proteins was expressed in arbitrary units (arb. units).

Morphological changes in the heart were estimated from the vascular index (S S vascular lumen/EA S section per 10,000 μ^2), cell index (S cell number/EA S section per 10,000 μ^2), width of muscle fibers (μ^2 in 30 fields of view), and necrotic area (μ^2 in 30 fields of view). Morphometry of heart samples was performed using a Quantimet 550IW video system (Leica) and Leica QWin16 software. Activities of type 1 lactate dehydrogenase (LDH_1) and creatine kinase (CK, mmol/mg tissue/min) in the myocardium were measured on an Ultrospec-4050 spectrophotometer.

The differences between the samples were evaluated by nonparametric Mann—Whitney *U* test. The differences were significant at $p<0.05$. The data are presented as the median and upper and lower quartiles (25th and 75th percentiles). The results were analyzed statistically using Statistica 6.0 software.

RESULTS

Cell transplantation in healthy animals was not followed by morphological and biochemical changes in the heart or variations in the concentration of heat shock proteins.

All stages of myocardial inflammation (*e.g.*, vascular, exudative, cellular, and proliferative compo-

TABLE 1. Effect of Cell Transplantation on Morphometric Characteristics of the Myocardium during Heart Injury

Period of study	Cell index, arb. units		Vascular index, arb. units		Cardiomyocyte area, μ^2	
	epinephrine	epinephrine+ CT	epinephrine	epinephrine+ CT	epinephrine	epinephrine+ CT
Control	—		0.021 (0.016-0.027)		212.6 (200.9-224.5)	
1 h	3.2	2.5	0.033	0.031	221.9	217.4
	(2.9-3.5)	(2.2-2.9)	(0.027-0.039)	(0.025-0.036)	(211.3-232.6)	(206.5-228.3)
4 h	8.3	6.1*	0.048$^+$	0.042$^+$	248.5	220.9
	(7.5-9.0)	(5.6-6.7)	(0.041-0.054)	(0.034-0.049)	(235.9-261.1)	(207.7-234.3)
8 h	14.7	10.2	0.061$^+$	0.047$^+$	268.7$^+$	238.8
	(12.6-16.7)	(8.9-12.5)	(0.056-0.066)	(0.042-0.053)	(255.3-282.3)	(226.1-251.7)
12 h	22.5	17.3	0.059$^+$	0.039^{+*}	291.3$^+$	257.3$^+$
	(20.0-24.9)	(13.6-21.1)	(0.052-0.065)	(0.033-0.047)	(275.4-307.2)	(245.6-269.1)
16 h	31.2	25.2	0.047$^+$	0.037	342.8$^+$	287.5^{+*}
	(27.4-35.0)	(20.5-30.1)	(0.040-0.056)	(0.029-0.042)	(327.9-357.8)	(271.7-303.5)
1 day	53.6	39.7	0.045$^+$	0.034	361.2$^+$	304.2$^+$
	(48.4-59.0)	(34.4-45.1)	(0.039-0.050)	(0.026-0.040)	(345.8-376.8)	(290.7-317.9)
3 days	69.1	58.7	0.046$^+$	0.033	389.1$^+$	332.8^{+*}
	(61.9-76.3)	(53.1-64.4)	(0.038-0.053)	(0.026-0.040)	(371.8-406.5)	(316.1-349.6)
7 days	46.2	22.3*	0.036	0.039$^+$	249.3	236.34
	(39.7-52.5)	(18.2-26.4)	(0.030-0.042)	(0.036-0.044)	(229.9-268.8)	(222.8-249.9)

Note. Here and in Table 2: data are presented as the median, upper quartile, and lower quartile. $p<0.05$: *compared to epinephrine-treated animals; $^+$compared to the control. CT, cell transplantation.

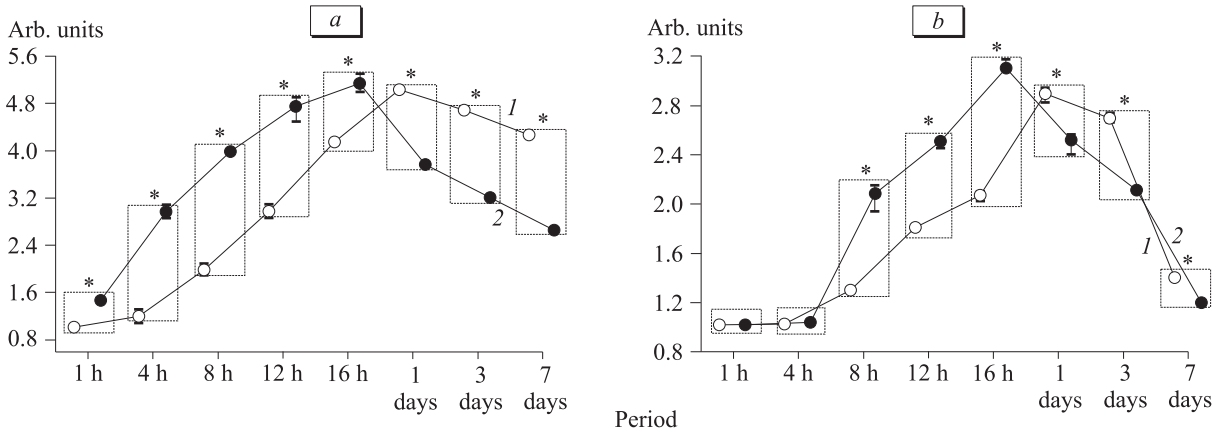

Fig. 1. Effect of cell transplantation on the amount of Hsp-70 (*a*) and Hsp-72 (*b*) in the heart of animals with epinephrine-induced myocardial injury. Here and in Figs. 2 and 3: epinephrine (*1*); epinephrine+cell transplantation (*2*). *$p<0.05$.

nents) were found in epinephrine-treated animals. The increase in the degree of the inflammatory response was accompanied by a rise in the vascular index, abundant leukocytic infiltration, and edema of myocardial fibers (Table 1). The vascular index reached maximum by the 8th-12th hour. The cell index and cardiomyocyte area were maximum on day 3 after epinephrine injection. The area of myocardial necrosis was also highest in this period (2069 [2046.4-2091.9] μ^2).

Transplantation of cell suspensions from the heart produced a strong effect on myocardial inflammation. Cell infiltration, vascular hyperemia, and edema of myocardial muscle fibers were less severe under these conditions. Macrophages and fibroblasts had a greater contribution to the qualitative composition of cell infiltrates in the myocardium of these animals (as compared to the previous series). The average area of myocardial necrosis in rats after transplantation of neonatal cardiac cells was much lower than in animals without transplantation. These differences were observed on days 1, 3, and 7 (by 10.14, 6.9, and 16.6%, respectively; $p<0.05$).

Activities of CK and LDH_1 in myocardial tissue were much higher after cell transplantation (compared to epinephrine-treated animals; Table 2).

The development of myocardial injury in rats of both groups was accompanied by similar changes in

TABLE 2. Effect of Cell Transplantation on Activities of LDH_1 and CK in the Myocardium during Heart Injury

Period of study	LDH_1		CK	
	epinephrine	epinephrine+CT	epinephrine	epinephrine+CT
Control	4.27 (4.20-4.34)	10.42 (10.23-10.61)		
1 h	3.26$^+$ (3.19-3.28)	3.07^{+*} (3.01-3.14)	7.87$^+$ (7.76-7.90)	7.97$^+$ (7.91-8.07)
4 h	3.11$^+$ (3.08-3.16)	3.54^{+*} (3.47-3.64)	7.97$^+$ (7.94-8.04)	8.36^{+*} (8.33-8.39)
8 h	3.55$^+$ (3.42-3.65)	3.96^{+*} (3.81-4.09)	9.18$^+$ (9.9-9.23)	10.35* (10.23-10.41)
12 h	3.65$^+$ (3.56-3.71)	4.46^{+*} (4.40-4.50)	6.12$^+$ (6.05-6.17)	7.78^{+*} (7.72-7.94)
16 h	3.14$^+$ (2.95-3.32)	4.98^{+*} (4.94-5.04)	5.64$^+$ (5.58-5.72)	7.1^{+*} (7.02-7.20)
1 days	1.47$^+$ (1.36-1.52)	3.37^{+*} (3.29-3.49)	7.88$^+$ (7.84-7.93)	10.43* (10.38-10.55)
3 days	2.59$^+$ (2.48-2.68)	4.32* (4.26-4.41)	7.53$^+$ (7.49-7.63)	11.51^{+*} (11.43-11.58)
7 days	3.35$^+$ (3.31-3.40)	3.85^{+*} (3.81-3.95)	5.78$^+$ (5.72-5.88)	9.32^{+*} (9.25-9.38)

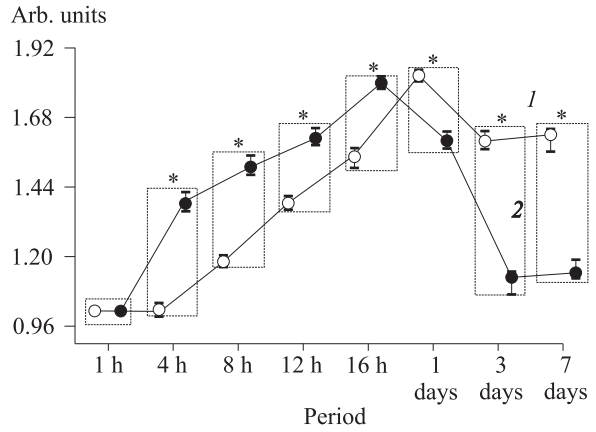

Fig. 2. Effect of cell transplantation on Hsp-60 concentration in the heart of animals with epinephrine-induced myocardial injury.

Hsp-70 concentration in the myocardium. However, some differences were found after cell transplantation.

Hsp-70 concentration in the myocardium of epinephrine-treated animals and rats of the cell transplantation group was highest 1 day and 16 h after the start of experiments, respectively (Fig. 1, *a*). Moreover, induction of Hsp-70 protein synthesis after cell transplantation was much more pronounced even at the early stage of myocardial injury (from the 1st hour). Transplantation was followed by increased production of not only stress proteins Hsp-70, but also inducible isoform Hsp-72 by heart cells. It should be emphasized that the increase in protein synthesis was observed by the 8th hour (not by the 1st hour; Fig. 1, *b*).

Changes in Hsp-60 concentration in the damaged heart coincided with the dynamics of Hsp-70 synthesis in animals of the cell transplantation group and epinephrine-treated rats (Fig. 2). The main difference was that the synthesis of Hsp-70 exceeded that of Hsp-60.

The accumulation of inducible sHsp-32 and sHsp-27 was studied during epinephrine-induced myocardial injury. The synthesis rate of sHsp-27 in the heart tissue increased more slowly than that of sHsp-32 (Fig. 3). The concentration of sHsp-32 was highest after 8 h and remained unchanged over the next 16 h. The concentration of sHsp-27 reached maximum on day 1 and remained unchanged for at least 2 days. Variations in the amount of sHsp-32 and sHsp-27 in the damaged myocardium did not differ in rats of the cell transplantation group and epinephrine-treated animals (Fig. 3). Comparative study showed that cell transplantation contributes to the increased intracellular synthesis of sHsp-32 (after 8 and 12 h) and sHsp-27 (after 12 and 16 h).

Our results indicate that cell transplantation accelerates and/or increases the induction of intracellular defense mechanisms in heart cells. These changes probably contribute to reduction of epinephrine-induced myocardial inflammation, maintenance of cardiomyocyte structure, and glycolysis.

Heat shock proteins play the major role in renaturation of denatured, abnormally folded, or aggregated protein, which restores functional activity of macromolecules [4]. The increase in the concentration of Hsp-70/72, Hsp-60, and small Hsp (*e.g.*, sHsp-27) prevents cell apoptosis [5], which is probably associated with the reparation or elimination of denatured proteins.

Published data show that implanted neonatal cells contain active antioxidants (vitamins A and E), microelements (activation of cellular antioxidant enzymes), glutathione, and glutathione-related enzymes [7]. Cytosolic proteins sHsp-32 and sHsp-27 play an important role in antioxidant protection of the cell during oxidative stress [5,11]. Small heat shock proteins of the sHsp-25/27 family inhibit actin polymerization [1,5], which prevents contracture of myocardial myofibrils after cardiac cells transplantation [7].

It remains unclear why the concentration of intracellular stress proteins increases in the earlier period after cell transplantation. It seems unlikely that this process is related to acceleration of cell reparation

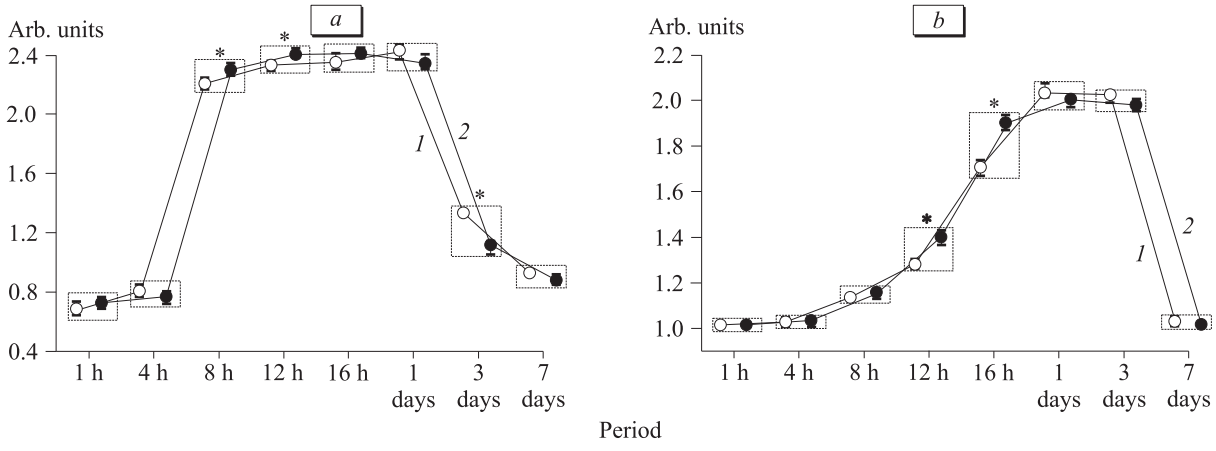

Fig. 3. Effect of cell transplantation on the amount of sHsp-32 (*a*) and sHsp-27 (*b*) in the heart of animals with epinephrine-induced myocardial injury.

in the myocardium (at least under these conditions). These changes are probably associated with the trigger action of transplant components on cytoplasmic or nuclear receptors on donor heart cells, which results in cascade activation of genes for the corresponding heat shock proteins. Further investigations should be performed to confirm this assumption.

We conclude that cell transplantation is followed by a rapid increase in the amount of stress proteins in heart cells after treatment with epinephrine. This procedure decreases the risk of myocardial injury.

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REFERENCES

1. N. B. Gusev, N. B. Bogacheva, and S. B. Marston, *Biokhimiya*, **62**, No. 5, 613-623 (2002).
2. I. Yu. Malyshev and E. V. Malysheva, *Byull. Eksp. Biol. Med*., **126**, No. 12, 604-611 (1998).
3. F. Z. Meerson and I. Yu. Malyshev, *Phenomenon of Adaptive Stabilization of Structures and Heart Protection* [in Russian], Moscow (1993).
4. N. K. Nagradova, *Sorosovsk. Obrazovat. Zh*., No. 7, 10-18 (1996).
5. O. O. Panasenko, M. B. Kim, and N. B. Gusev, *Uspekhi Biol. Khim*., **43**, 59-98 (2003).
6. V. S. Repin and G. T. Sukhikh, *Medical Cell Biology* [in Russian], Moscow (1998).
7. A. A. Runovich, V. K. Voinikov, Yu. I. Pivovarov, *et al*., *Atherosclerosis and Cell Therapy* [in Russian], Irkutsk (2005).
8. Yu. L. Shevchenko, A. S. Svistov, V. V. Tyrenko, *et al*., *Fiziol. Chel*., **25**, No. 1, 134-139 (1999).
9. M. E. Feder and G. E. Hofmann, *Annu. Rev. Physiol*., **61**, 243-282 (1999).
10. D. Hess, L. Li, S. Sakano, *et al*., *Nat. Biotechnol*., **21**, No. 7, 763-770 (2003).
11. S. W. Ryter, J. Alain, and A. M. K. Choi, *Physiol. Rev*., **86**, No. 2, 583-650 (2006).
12. Q. Xu, G. T. Schett, C. Li, *et al*., *Circ. Res*., **86**, No. 11, 1122-1128 (2000).