Transplantation of Xenogenic Cardiomyocytes in Experimental Epinephrine-Induced Damage to Myocardium: Enzyme Activity and Morphological Parameters

S. L. Bogorodskaya, S. N. Clinova, M. B. Mikashova, S. S. Golubev, J. I. Pivovarov, T. E. Kurilskaya, and A. A. Runovich

Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 3, pp. 132-135, August, 2008
Original article submitted February 21 18, 2008

We studied the effect of xenogenic neonatal cardiomyocytes on enzymatic processes in rat myocardium under conditions of epinephrine-induced damage. It was found that transplantation of the cell preparation restricts the zone of suppressed enzyme activity in the myocardium and promotes its recovery. Less pronounced metabolic disturbances corresponded to less pronounced morphological changes in the myocardium.

Key Words: *myocardium; enzymes; epinephrine; cell transplantation*

It is now established that cell therapy accelerates repair processes in damaged organs, including the myocardium. It was shown that cell preparations limits the zone of damage, improved cardiac function, stimulate angiogenesis [1,4,6,9,14], improve myocardial perfusion [4,12], and suppress myocardial remodeling; the possibility of cardiomyocyte regeneration are now studied [2,10,13].

Here we studied the effect of cell therapy on metabolic processes in the myocardium, in particular, on activity of enzymes involved into the synthesis, transport, and utilization of ATP: lactate dehydrogenase 1 (LDH_1), creatine kinase (CK), and ATPase.

MATERIALS AND METHODS

Experiments were carried out on outbred male rats weighing 250-300 g. Epinephrine stress was modeled by single subcutaneous injection of 0.1% epinephrine in a dose of 0.5 mg per 100 g body weight. Group 1 animals (n=85) received physiological saline immediately after epinephrine; to group 2 animals (n=88), isolated cardiomyocytes from newborn rabbits were injected subcutaneously (500,000 cells in 0.5 ml physiological saline). Initial parameters were measured in 6 healthy rats.

The blood for biochemical tests were taken 1, 4, 8, 12, 16, and 24 h and on days 3 and 7 after administration of epinephrine. Activity of CK was measured using Biocon kits. LDH_1 activity was evaluated by its α-hydroxybutyrate dehydrogenase activity using Cormay kits. Total activity of ATPases was evaluated by accumulation of inorganic phosphorus. Measurements were performed on Ultrospec-4050 spectrophotometer and Roki semiautomated biochemical analyzer. Light microscopy of myocardial samples was performed using Quantimet 5501 W computer-assisted video system.

The data were processed statistically by Student t test and Mann—Whitney test using Statistica software. The differences were significant at $p<0.05$.

Research Center of Reconstructive and Repair Surgery, East Siberian Research Center, Siberian Division of Russian Academy of Medical Sciences, Irkutsk. ***Address for correspondence:*** bogorodskaya@mail.ru. S. L. Bogorodskaya

0007-4888/08/1461-0120

RESULTS

In the myocardium of control rats, LDH_1 activity decreased 1-4 h after the start of the experiment (Fig. 1, *a*). This period corresponded to maximum hypoxic suppression of enzyme activity. In experimental animals receiving injection of cardiomyocytes, minimum activity of LDH_1 was observed in the sample taken after 1 h. Then, a tendency towards earlier recovery of enzyme activity compared to the control group was observed: in animals receiving cardiomyocytes, this parameter was higher starting from the 4th hour. The maximum values surpassed the control, which attests to not only recovery, but also activation of LDH_1. In the group without transplantation, LDH_1 activity did not return to the initial level. Thus, early recovery of LDH_1 activity in the myocardium indicates more favorable conditions for enzyme functions (less hypoxic). It is obvious, that the dynamics of activity of glycolytic enzyme LDH_1 corresponded to shorter period of suppression of glycolytic processes, their earlier recovery and activation.

Activity of CK in the myocardium decreased during the first hours of the experiment. This recovery occurred earlier in the group with cell transplantation: similarly to LDH_1 activity, activity of CK by the 4th hour of the experiment was considerably higher than in the control group (Fig. 1, *b*). At later terms of the experiment, CK activity in the group with cell transplantation returned to the initial level and were higher than in the group without transplantation. Thus, CK system of phosphate transport to the sites of ATP resynthesis and utilization (ATPases of myofibrils, sarcoplasmic reticulum, and sarcolemma) recovered earlier and was more active after cell transplantation.

Summary ATPase activity decreased 4 h after epinephrine injection (Fig. 1, *c*). At this stage, ATPase activity in the experimental group significantly surpassed that in the control group and hence, its hypoxic suppression was less pronounced. Further recovery was more intensive and maximum values were observed after 4 h. In the control group, ATPase activity recovered at later terms and maximum activity was observed on day 3. Higher level of ATPase activity in the myocardium observed in most animals with transplanted cardiomyocytes attests to less pronounced disturbances and earlier recovery of active ionic transport across the cell membranes which is a prerequisite for functional integrity of the cell.

Morphological findings confirm less pronounced damage to the myocardium after cell transplantation.

The first foci of necrosis were found by 16th hour in the experimental group and by the 12th hour in the control group. The summary area of necrotic foci in the group with transplantation was significantly lower and a tendency towards their more rapid decrease was observed at later terms (Table 1).

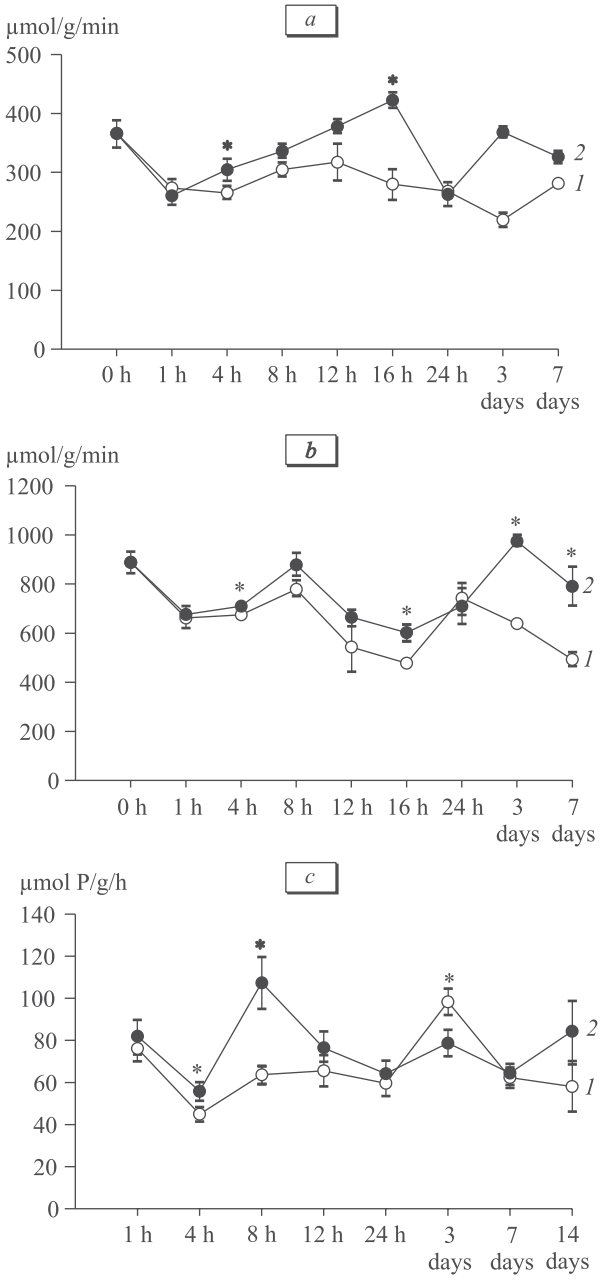

Fig. 1. Effect of Xenogenic Neonatal Cardiomyocytes on LDH_1 (*a*), creatine kinase (*b*), total ATPase (*c*) activities in rats with epinephrine-induced damage to the myocardium. *1*) subcutaneous injection of epinephrine in a dose of 5 mg/kg body weight and 0.5 ml physiological saline; *2*) subcutaneous injection of epinephrine in a dose of 5 mg/kg body weight and 500,000 xenogenic cardiomyocytes in 0.5 ml physiological saline. *$p<0.05$ compared to the control.

TABLE 1. Morphological Parameters of the Myocardium in Control (Epinephrine+Physiological Saline, *n*=54) and Experimental Rats (Epinephrine+Xenogenic Cardiomyocytes, *n*=53, *M*±*m*)

Term of experiment	Vascular index (S vascular cross-section/S section)		Total area of necrotic foci in field of view at ×200, μ²		Cardiomyocyte cross-section area, μ²	
	control	experiment	control	experiment	control	experiment
Initial value	0.021±0.005	—	212.61±11.83			
1 h	0.033±0.006	0.031±0.006	—	—	221.93±10.65	217.42±10.91
4 h	0.048±0.007	0.042±0.007	—	—	248.52±12.59	220.95±13.32
8 h	0.061±0.005	0.047±0.006*	—	—	268.77±13.46	238.89±12.83
12 h	0.059±0.006	0.039±0.006*	20.6±4.7	—	291.3±15.91	257.32±11.76
16 h	0.047±0.007	0.037±0.005	56.5±7.3	42.9±6.1	342.81±14.95	287.59±15.89*
1 day	0.045±0.005	0.034±0.006	572.7±12.4	514.3±13.5*	361.28±15.53	304.28±13.62*
3 days	0.046±0.008	0.033±0.007	2069.4±22.9	1926.7±18.6*	389.12±17.34	332.85±16.73*
7 days	0.036±0.006	0.039±0.004	1634.2±17.4	1362.7±19.3*	249.32±19.49	236.34±13.52

Note. *$p<0.05$ compared to the control.

Degenerative changes in cardiomyocytes were less pronounced: the cross-section area of muscle fibers was lower after transplantation.

Acute damage was primarily associated with vascular plethora. The corresponding vascular index (the ratio of summary vascular cross-section area to total area of the section) was higher after cell transplantation (Table 1). Thus, enzyme activity in the myocardium was lower in animals without transplantation compared to rats after transplantation: LDH_1 and CK activities did not return to the initial level, while ATPase activity recovered later. This attest to a state similar to myocardial stunning [5]. Thus term was proposed for the state of postischemic dysfunction of the left ventricle persisting after reperfusion despite resumption of coronary blood flow and the absence of irreversible changes in the myocardium. In our experiment, the myocardium was damaged and the severity of damage was different in the control and experimental groups: the total area of necrotic foci at different terms after transplantation decreased by 10-20% (Table 1). However, differences in enzyme activities were more pronounced: in animals with transplantation, LDH_1 activity was higher by about 50% after 16 h and ATPase activities were higher by more than 60% after 8 h. It is known that impairment of metabolic and contractile activity of the myocardium is determined by reduced ATP content due to enhanced hydrolysis and impaired synthesis of ATP. Increased content of adenosine reduces the sensitivity of adrenoceptors, accumulation of inorganic phosphate and developing acidosis reduce the sensitivity of myofibrils to calcium ions, while the loss of potassium ions decreases excitability. These changes shift adrenergic and noradrenergic regulation of metabolic processes towards cholinergic one and modulate NADH/NAD ratio, the deficit of HAD and acidosis lead to inhibition of enzyme activities [3,7,8].

Inhibition of metabolic processes in the myocardium is an adaptive mechanism aimed at maintenance and prolongation of reversibly damaged cardiomyocytes via saving energy resources and prevention of their exhaustion; this limits cell damage and saves reserves for further recovery. However, stunning and hibernation of the myocardium can underlie the development of heart failure. Stunning leads to dysfunction of the left ventricle and then with aggravation of structural changes to heart failure. The severity and duration of metabolic stunning depend on the degree and duration of stress and ischemia. Hence, we can hypothesize that in animals with transplanted cardiomyocytes these processes are less pronounced and long-lasting.

Thus, the following conclusions can be made. Transplantation of xenogenic neonatal cardiomyocytes in epinephrine-induced damage to the myocardium limited stress/ischemia-induced metabolic stunning of the myocardium and more rapid and complete recovery of enzyme activities.

This procedure also prevented inhibition and promoted recovery of activity of membrane ATPase proteins, and hence, to more rapid restoration of ion transport across the membrane.

Early recovery of LDH_1 activity after transplantation implies more optimal conditions for its functioning and higher activity of glycolytic processes in this group. CK system of ATP phosphate transport also underwent less pronounced inactivation and recovered earlier after cell transplantation.

Less pronounced metabolic disturbances in the myocardium after transplantation of xenogenic neo-

natal cardiomyocytes corresponded to less pronounced morphological changes; in particular, hemodynamic disturbances, degenerative, and necrotic processes were less severe.

REFERENCES

1. *Atherosclerosis and Cell Therapy*. Ed. A. A. Runovich, J. I. Pivovarova, and T. E. Kurilskaya [in Russian], Irkutsk (2005).
2. Yu. N. Belenkov, F. T. Ageev, V. Yu. Mareev, *et al., Serdechn. Nedostat.*, **4**, No. 4, 168-173 (2003).
3. M. V. Bilenko, *Ischemic and Reperfusion Damage to Organs* [in Russian], Moscow (1989).
4. L. A. Bokeriya, Yu. I. Buazishvili, S. T. Matskeplishvili, *et al., Kardiologiya*, No. 9, 16-22 (2004).
5. A. S. Bushmelev, *Serdechn. Nedostat.*, **3**, No. 6, 318-321 (2003).
6. A. E. Vermel, *Klin Med.*, **82**, No. 1, 5-11 (2004).
7. P. F. Litvitskii, *Pat. Fiziol. Eksp. Ter.*, No. 2, 2-12 (2002).
8. M. G. Pshennikova, *Ibid.*, No. 4, 21-31 (2000).
9. V. I. Shumakov, E. N. Kazakov, N. A. Onishchenko, *et al., Ros. Kardiol. Zh.*, No. 5, 42-50 (2003).
10. J. M. Hill, D. Orlic, and A. E. Arai, *Circ. Res.*, **91**, No. 12, 1092-1102 (2002).
11. Huang H.-Y., Huang Z., Hou J. *et al., Acad. J. Second Mil. Univ.*, **25**, No. 9, 950-955 (2004).
12. C. T. Mesquita, M. C. P. Pessoa, E. Perin, *et al.,* Eur. J. Nucl. Med. Mol. Imag. **29**, Suppl. 1, 226 (2002).
13. C. E. Murry, *Acta Histochem. Cytochem.*, **35**, No. 3, 210 (2002).
14. T. Ueno, T. Murohara, K. A. Robinson, *et al., Boston Scientific Scimed, Inc.* N 09/915853 (2005).
15. Z. M. Zhu, L. R. Gao, Y. X. Fei, *Acad. J. Second Mil. Univ.*, **25**, No. 9, 936-939 (2004).