The Energy Supply Systems in the Heart Valve Leaflet Cells during Kryopreservation and Storage

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The condition of animal heart valve allografts after kryopreservation and storage is assessed. After 2-day storage of preparations at 4°C tissue ATP content decreases by 1.5-2 times and remains at this level for 5 days. Freezing of tissues without kryoprotector results in more than 10-fold drop in ATP content and a 2-fold decrease in the total content of pyridine nucleotides. After kryopreservation these parameters decrease by 1.2-1.4 times.

Key Words: *cardiac valve allografts; kryopreservation; energy supply systems*

Allografts of the aorta and lung stem kryopreserved without carcass are widely used in surgical treatment of left- and right-ventricular valvular defects. Preservation of allograft cells is an important factor in prolongation of the terms of normal function of transplanted valves [11]. The use of viable allografts involves an operative assessment of their intactness and viability during procurement, kryopreservation, and storage. Previously, the status of cardiac valve leaflet cells (CVLC) was assessed by the number of intact cells, as determined by incorporation of labeled amino acids in proteins [9,10], and tissue viability was estimated by cell growth in culture [4]. Since both methods are labor- and time-consuming, new approaches to rapid analysis of tissue status have been developed.

Cell energy systems and transformation of energy in the mitochondria (MC) are sensitive to environmental factors, such as low temperature and kryoprotective agents [2,3]. Mitochondrial injury is one of the initial key stages of cell necrosis in oxidative and toxicological stress [7,12,13]. Mitochondrial function is greatly suppressed in ischemia and perfusion of organs, including the myocardium [6].

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MATERIALS AND METHODS

Porcine cardiac valve cusps were used. After sacrifice, the tissues were put in RPMI-1640 and cooled to 4°C. Tissues were sterilized by several-hour incubation at 4°C in the same medium with the antibiotics cephalosporin (2.5 g/liter), ampiox (2.5 g/liter), gentamycin (0.4 g/liter), and claforane (0.5 g/liter). For measuring ATP and pyridine nucleotides (PN), tissue fragments (100 mg) were incubated for 10 min at 37°C, fixed in liquid nitrogen, grind in a mortar cooled with liquid nitrogen, transferred into 0.5 ml extracting solution (20 mM phosphate buffer, pH 7.0 for ATP and pH 8.5 for PN), heated to 95°C, extracted for 2 min at 95°C, and rapidly cooled to 4°C. The content of ATP and PN was measured by the chemiluminescence method in a Lucifer-2 chemiluminometer using ATP reagent (Microlyum, Chemical Faculty of the Moscow University) [1] and NADPH reagent (KRAB, Institute of Biophysics, Siberian Division of Russian Academy of Sciences) [8]. Oxidized PN were reduced in the glutamate-glutamate dehydrogenase system. The rate of oxygen

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consumption was estimated with a Clark's electrode [5] at 37°C in RPMI-1640. For kryopreservation the tissue was put in 10% dimethyl sulfoxide in bovine serum, left at 20°C for 10 min, rapidly cooled to 4°C in the same solution, and then to -40-45°C at a rate of 1-2°C/min. The containers with the preparations were put in liquid nitrogen. For defrosting the containers were put in water at 37°C, rapidly (1-3 min) washed from dimethyl sulfoxide, and put in RPMI-1640. Rapid freezing in liquid nitrogen without kryoprotector followed by defrosting damaged CVLC. Mean data of 3-5 experiments are presented.

RESULTS

CVLC intactness was assessed by the total content of ATP and PN and the degree of NADP reduction in intact tissues and in tissues frozen under different conditions. Table 1 shows that the content of ATP and total PN (NADP and NADPH) is increased in preparations cooled to 4°C. After injury, the content of ATP drops 10-fold, the content of total PN drops more than 2-fold, and the degree of their reduction decreases. The NADPH/NADP ratio in cooled preparations is 1.53 *vs.* 0.78 in damaged ones. After kryopreservation of cardiac valves, the parameters characterizing the status of energy supply systems and, therefore, tissue intactness, were much higher than after rapid freezing without kryoprotector: the content of ATP and total PN decreased only by 1.5-1.6 times.

Storage of intact sterilized preparations at 4°C resulted in a 1.3-fold decrease of ATP content during the first 2 days of storage which then stabilized (Fig. 1, *1*). This indicates a 25% cell death in a preparation during the initial period of storage.

After kryopreservation followed by storage at 4°C the time course of ATP content was similar (Fig. 1, *2*). The level of ATP in the cells remains rather high during the first 2 days of storage of frozen (at -20°C) preparations, but later it decreases (Fig. 1, *3*).

Succinate stimulation of respiration (increased cellular permeability for succinate, succinate does not penetrate in intact cells) and a decrease in respiratory

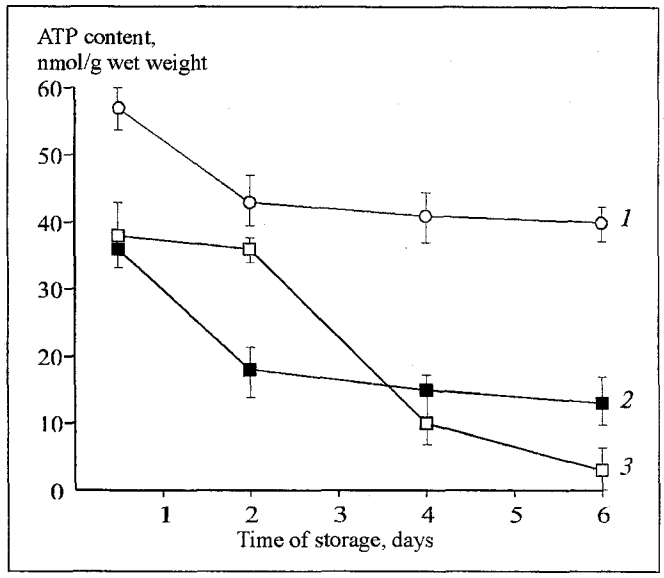

Fig. 1. Changes in ATP content in cardiac valve leaflet cells after storage under different conditions. *1*) intact preparations stored at 4°C; kryopreserved preparations stored at 4°C (*2*) and -20°C (*3*).

rate (loss of endogenous substrates by cells) indicate impaired permeability of plasma membranes. Table 2 shows the mean rate of oxygen consumption by intact (cooled), kryopreserved, and damaged (frozen without kryoprotector) preparations. Storage at 4°C for 4 days and sterilization in antibiotic solution for 1 day did not decrease the rate of cell respiration and did not enhance succinate stimulation of respiration.

Tissue content of ATP and NADPH proved to be a sensitive indicator of CVLC intactness. A decrease in the content of ATP indicates its hydrolysis and inhibition of energy-dependent processes in cells. ATP content in cells and tissues of an organism is normally maintained at a level of 3-5 mM due to equilibrium of ATP production and consumption. If membrane permeability is impared, ATP production in MC is decreased or completely inhibited, and as a result, the level of ATP drops and PN are oxidized. Moreover, cells can lose ATP and PN as a result of impaired permeability of plasma membrane. The proposed methods for assessing CVLC intactness

Table 1. Changes in the Content of ATP and PN in CVLC (nmol/g wet weight) after Different Treatments (*M±m*)

Parameters	Treatment of preparations		
	cooling to 4°C	freezing without kryoprotector	kryopreservation
ATP	59.9±4.5	0.48±0.12	37.1±4.3
NADPH+NADP	18.1±1.6	7.5±0.8	12.3±1.4
NADPH	11.2±0.8	3.3±0.4	Not measured
NADPH/NADP	1.53	0.78	-"-

Table 2. Rate of Oxygen Consumption by CVLC (pmol O_2/min×mg wet weight tissue) after Different Treatments ($M\pm m$)

Additives	Treatment of preparations		
	cooling to -4°C	freezing without kryoprotector	kryopreservation
No additives	25.0±4.5	7.0±4.4	22.2±4.9
Succinate, 10 mM	27.2±5.0	36.4±5.5	28.6±6.1

permit the detection of impaired permeability and injury to cellular (by decreased total content of PN) and intracellular, specifically, mitochondrial membranes (by decreased ATP level and degree of PN reduction). These methods for assessing CVLC viability can be used for searching the optimal conditions of kryopreservation and storage of allografts and for assessing the status of preparations used for organ transplantation, because the time of ATP analysis is less than 30 min and it can be carried out with a small amount of tissue.

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