Use of Choline Chloride for Leukocyte Cryopreservation (-40°C)

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Choline chloride in complex with the main cryophylactic (1,2-propanediol) preserves morphological integrity and functional activity of blood nuclears after freezing to -40°C by the exponential program.

Key Words: *leukocytes; choline chloride; 1,2-propanediol; cryoprotective solution; phagocytosis*

The search for new substances with cryoprotective properties remains an important problem of cryobiology. It was shown that cryoprotective effects of substances are largely determined by their capacity to bind water and modify the structure of solutions so that they froze in an amorphous state. This prevents excessive dehydration, one of the priority factors of cell membrane cryodamage [1]. The results obtained in Kharkov Institute of Cryobiology and Cryomedicine [3] indicate that choline chloride possesses these physicochemical properties: due to its charged group and hydroxyl groups it binds water. Phase transitions and physical states of aqueous solutions of choline chloride and its cryoprotective effects towards erythrocyte membranes were studied. It was proven that the water-choline chloride system is characterized by the common property of the cryoprotective systems, high liability to solidification, which indicates sufficiently strong water binding by choline chloride. However, erythrocytes contain no nuclei and are sufficiently resistant to osmotic shock, in contrast to leukocytes, nuclear cells with intensive metabolism and poor resistance to any stress exposure. Therefore, our aim was to evaluate the efficiency of choline chloride for freezing blood nuclear cells (leukocytes) to -40°C by the exponential program.

MATERIALS AND METHODS

The study was carried out on 10 donor concentrated leukocyte suspensions obtained from donor whole blood by cytopheresis. The mean volume of biological object was 20.0±3.5 ml. Concentrated leukocytes were mixed (1:1) with one of 5 cryoprotective solutions (Table 1) in a Compoplast 300 plasticate container and exposed at ambient temperature during 20 min.

The main component of all variants of cryoprotective solution is 1,2-propanediol (endocellular protector). In addition, each variant included choline chloride. Choline chloride, a representative of group B vitamins; it is characterized by membrane-protective, lipotropic, antidepressant effects. Other components were hydroxyethylstarch (HES) with weak exocellular effects, requiring no washing after biological object warming and used in Russia as transfusion medium; hydroxymethylethylpyridine succinate (HOMEPS), a drug with antioxidant, antihypoxic, membrane-protective, stress-protective effects; glucose with slight exocellular effects, which served largely as an energy substrate in this solution. The optimal 1% concentration of choline chloride was selected in previous studies. It was used for preparation of all variants of cryoprotective solution.

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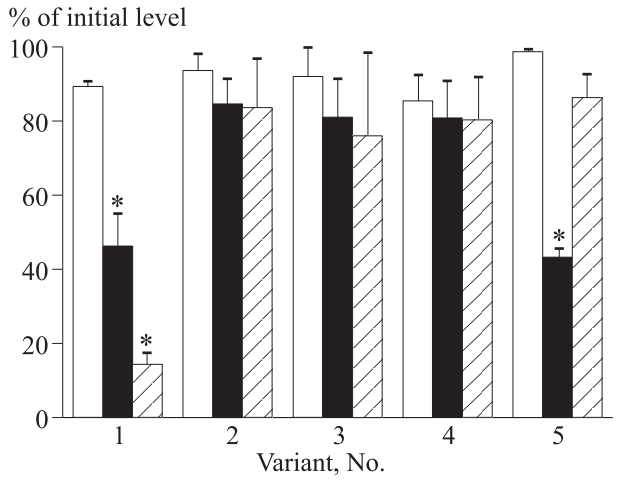

Fig. 1. Leukocyte preservation after 24-h anabiosis at -40°C in different cryoprotective solutions. Light bars: leukocyte count; dark bars: viability; cross-hatched bars: granulocyte count. Number of observations: 7. *p<0.05 compared to variants Nos. 2, 3, 4.

After detecting the most effective variant of solution, two additional series of experiments were carried out: with cryoprotective solution containing only 1,2-propanediol in the final concentration of 21% (series I) and with cryoprotective solution of the optimal composition without choline chloride (series II).

The cells were frozen according to the exponential program in three stages: first in ethanol (96°C) bath in a Cryostat electric freezer (-28°C, 15 min), than in the freezer (-40°C). Freezing rate at stage 1 was 8-10°C/min to eutectic point (-11.5°C), at stage 2 it was 1-2°C to -28°C, and at stage 3 it was 0.3-0.6°C/min to - 40°C.

Rapid defrosting of concentrated leukocytes was carried out after 24-h storage of the object at -40°C in a 20-liter water bath, warmed to 38°C, over 45-60 min with intense shaking of the container.

Native and defrosted leukocytes were counted in a Goryaev chamber, their morphological composition was evaluated (differential count), barrier characteristics of leukocyte plasma membranes were determined in the eosin exclusion test [5], and phagocytic activity of neutrophils was evaluated using inert particles (latex) [4]. The data were processed statistically using Wilcoxon's test [2].

RESULTS

Variants Nos. 2, 3, and 4 of cryoprotective solution preserved the leukocyte structure during freezing/defrosting significantly better (p<0.05) than variants Nos. 1 and 5 (Fig. 1). Variant No. 4 exhibited the best

TABLE 1. Final Concentration of Components (%) of Cryoprotective Solution Used for Leukocyte Freezing to -40°C

Component	Variant of cryoprotective solution				
	No. 1	No. 2	No. 3	No. 4	No. 5
1,2-propanediol	15	18	19.5	21	24
Choline chloride			1		
HOMEPS			0.2		
Glucose			3		
HES			up to 200 ml		

TABLE 2. Comparative Analysis of Results of Experimental Series I and II ($M\pm m$)

Parameter	Relative number of leukocytes, % of initial level		
	series I	series II	control (variant No. 4)
Cell content	64.2±16.1	88.1±10,3*	89.6±9.2*
Eosin resistance	75.0±10.2	92.0±5.8*	80.9±9.3
Granulocytes	39.2±9.8	31.8±3.9	77.9±11.2*
PAN	Poor resistance to incubation at 37°C		66.4±5.2

Note. Number of observations: 7; *p<0.05 compared to series I.

cryoprotective effect for blood nuclears (final concentration of the protector 1,2-propanediol was 21%). Only after the use of this solution was it possible to evaluate the phagocytic activity of neutrophils (PAN) after defrosting. This parameter was 52.4±2.2% in native neutrophils and 35.0±4.0% in defrosted cells. In other experiments (solutions Nos. 1, 2, 3, and 5), the neutrophil resistance to incubation at 37°C (needed for PAN evaluation) was poor, leading to their rapid death.

Comparison of variant No. 4 (taken as control) with experimental series I and II (Table 2) showed that the basic cryophylactic 1,2-propanediol had a favorable impact on blood nuclears only when it was used in complex with restoring additives. However, the cryoprotective effect increased significantly with addition of choline chloride, which can be explained by its capacity to underact with cell membranes through exchange of bound water, reduce mechanical tension of membranes and level the restructuring of the lipid-lipid and lipid-protein complexes. Hence, addition of choline chloride to cryoprotective media promotes better preservation of blood nuclear cells at -40°C.

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