EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Creation of a Gelatin-Based Solution for Preservation of Leukocytes

E. P. Svedentsov, E. S. Stepanova, T. V. Tumanova, O. O. Zaytseva, and O. N. Solomina

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A new effective and available method is proposed for preservation of human blood leukocytes at supercooling temperature (-10°C). The method consists in induction of cold hypobiosis under protection of an original antifreeze protecting solution containing glycerol (cryoprotector), mexidol (antioxidant and membrane stabilizer), and gelatin preparation (modegel or gelatinol) as the solvent. No washing of the solution from the biological object before its intravenous injection is needed; the solution preserves morphological integrity of cells and their functional activity at a high level for 12 days.

Key Words: *leukocytes; cold hypobiosis; cold-resistant preserving solution; mexidol; gelatin preparations*

Transfusion of leukocytic concentrates is an effective method for prevention and treatment of many infectious complications resistant to antibiotic therapy [3,5,7,11]. However, rapid and significant exhaustion of the metabolic processes in leukocytes at positive temperatures [1,6,7] impedes their storage. The most effective methods for programmed cryopreservation with liquid nitrogen as the coolant preserve viability of just 70-80% cells [1]. Therefore, the development of new methods for preservation of the nuclear blood cells is a priority problem of cryobiology and transfusiology, while creation of cold-resistant preserving solutions is one of the stages in solution of this problem.

The aim of this study was creation of an effective antifreeze preserving solution, needing no washing from the biological object and sparing the morphological structure and functions of human blood leukocytes after their recovery from cold hypobiosis.

Laboratory of Blood Cryophysiology, Institute of Physiology, Komi Research Center, Ural Division of Russian Academy of Sciences, Syktyvkar. ***Address for correspondence:*** stepanova-1@yandex.ru. E. S. Stepanova

MATERIALS AND METHODS

The study was carried out on leukocyte concentrates (LC) prepared from 300 ml whole blood of volunteers by cytapheresis (Sorvell, 2500*g*, 5 min with cooling; citroglucophosphate as the preserving agent); the LC volume was 25.2±2.2 ml, cell count (19,610±6656)×10^{12}/ml.

Before cooling LC were mixed (1:1) with one of two variants of original cold-resistant preserving solution in a Compoplast 300 plasticate container. The resultant mixture was exposed at ambient temperature for 20 min, after which the container with the biomaterial was plunged into the Kriostat electric freezer tank (4000 ml), cooled to -10°C and filled with cold carrier (96% ethanol). The leukocytes were introduced in hypobiosis by the exponential mode of cooling. The biological object was then transferred for storage into a hermetically clo-

sed vessel filled with 45% ethanol (-10°C), which was placed into the electric freezer (-10°C). The preparation was warmed after 1, 5, 7, 9, 11, 12, and 14 days in a water bath (10 liters) at 38°C over 2-4 h with intense shaking (3-4 shakes/sec).

The main component of the preserving solution for human blood LC at -10°C is glycerol (endocellular cryoprotector); its colligative effect stabilizes the adjacent water molecules and reduces the temperature of the solution freezing. This protector was used in a low nontoxic concentration, and hence, an additional component was needed to potentiate the protective effect and maintain the cells suspended. Gelatin preparations were used for this purpose (gelatin preparations are characterized by a slight exocellular cold-resistant effect and are used as plasma substitutes) [4,10]. Mexidol was added to the solution as a membrane stabilizer, antioxidant, and antihypoxant, in order to eliminate the destructive effects of hypometabolic processes running at -10°C. Two variants of preserving solution with the same concentrations of mexidol and glycerol were created; in one gelatinol served as the solvent, in the other modified gelatin solution — modegel, with a lesser molecular weight and isoosmolar for blood plasma. Anticoagulants were also added, more potent into solution with gelatinol because of calcium ions it contained.

Leukocyte morphology and functions were studied before cooling and after warming. The cells were counted in Goryaev's chamber, quantitative composition of leukocytes was evaluated in smears stained by May—Grunwald and Romanowskii's methods, blood nuclear cell viability was evaluated in samples free from eosin [9]; neutrophil and monocyte phagocytic activity (PA; percentage of phagocytic cells in the total cell count) [9] and the neutrophil and monocyte phagocytic index (PI; number of latex particles, 1.03 μ in diameter, phagocytosed by the cell) were determined. Oxygen-dependent bactericidal mechanism of neutrophils was evaluated by the NBT test [9].

The significance of differences between the parameters was evaluated using Student's paired test [2].

RESULTS

The solution containing modegel promoted the preservation of a greater number of leukocytes after 12-day storage at -10°C than solution with gelatinol ($p<0.05$; Table 1).

Study of the morphological composition of leukocytes (Table 1) showed that on day 7 gelatinol-containing solution more effectively preserved granulocytes than the solution with modegel ($p<0.05$). However, as soon as on day 9 and later the efficiency of cryprotection by the modegel-containing solution became higher ($p<0.05$).

Evaluation of eosin resistance of leukocytes (Table 1) showed that the number of viable cells on day 11 of LC storage was significantly higher ($p<0.05$) in modegel-containing solution and on day 14 in gelatinol-containing solution.

Evaluation of PA and PI showed that phagosomes containing phagocytosed latex particles formed during all periods of the biological object

TABLE 1. Morphofunctional Characteristics of Human Blood Leukocytes Stored at -10°C in two Variants of Cold-Resistant Preserving Solution Containing Gelatin Preparations, Mexidol, and Glycerol (% of Initial Values; *M*±*m*; *n*=7)

Duration of storage, days	Modegel-containing solution	Gelatinol-containing solution
Leukocyte content		
1	95.2±6.4	98.4±2.0
7	88.2±8.7	92.7±7.3
9	90.4±9.9	84.8±10.9
11	88.8±10.3	80.7±8.9
12	92.6±4.2	84.1±4.5*
14	92.8±8.6	80.4±2.9*
Granulocyte content according to leukocytic formula		
1	88.4±6.4	89.6±12.3
7	83.6±5.8	95.3±6.6*
9	88.0±9.3	77.2±8.8*
11	81.7±5.5	68.0±4.6*
12	82.2±3.6	63.2±7.7*
14	81.4±4.5	62.8±6.9*
Eosin resistance		
1	91.3±5.5	97.2±3.2
7	91.1±8.9	86.8±4.7
9	86.9±11.2	83.5±6.0
11	91.4±4.7	82.7±6.8*
12	81.3±4.6	76.6±7.7
14	56.6±7.8	74.7±12.7*
Neutrophil PA		
1	98.9±1.6	88.4±7.0*
7	94.8±4.8	78.5±2.7*
9	76.7±5.0	72.6±3.8
11	75.5±9.8	61.4±2.8*
12	75.7±4.3	57.2±4.4*
14	52.9±6.1	45.5±3.0*

Note. $p<0.05$ compared to modegel-containing solution.

storage at -10°C in both variants of the preserving solution. The modegel-containing variant of preserving solution more effectively ($p<0.05$) protected neutrophil PA (Table 1) and PI starting from day 1 of cryohypobiosis: the mean number of latex particles absorbed by neutrophils before cooling was 10, after 1 day of cold hypobiosis 8 and 4, and after 12 days 4 and 2 after storage in modegel- and gelatinol-containing solutions, respectively. Monocyte PA and PI virtually did not change during the entire period of LC storage with both variants of cold-resistant preserving solution. Monocyte PA, initial and after cold hypobiosis of leukocytes, was about 2% with 2 latex particles per cell during all periods of storage.

No appreciable differences between the results of storage in two variants of solution were detected for oxygen-dependent biocidal capacity in the NBT test. The number of NBT-positive cells increased 1.5 times on day 12 and 2-fold on day 14 of storage in both cases.

The results indicate that optimal duration of LC storage at supercooling temperature with two variants of solution is 12 days. The modegel-containing solution exhibited better cryoprotective characteristics over this period [8]. The proposed cold-resistant preserving solution can be used in biological studies requiring preservation of animal cells and, after clinical trials, in transfusiology.

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