A New Method for Preservation of Viable Leukocytes at Near-Zero Temperatures

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 9, pp. 358-360, September, 2007
Original article submitted November 27, 2006

We propose a new effective, available, and economic method for preservation of leukocytes at near-zero temperatures for 6 days using an original protecting solution.

Key Words: *leukocytes; about-zero temperatures; protecting solution; phagocytosis*

Long storage of leukocytes in a biologically intact state at positive temperatures is difficult because of their rapid metabolism and rapid exhaustion of energy potential. The viability of leukocytes at 4°C can be maintained for up to 24 h [4]. The viability of granulocytes decreases first, because these cells are characterized by more intricate morphological organization. Condensation of nuclear membrane, partial lysis of mitochondria, and vacuolation of cytoplasm take place in these cells within 48 h [3,5]. Hence, in order to maintain leukocyte viability of at temperatures 0-2°C, a protecting solution (PS) is needed, which would preserve cell reserves and promote rapid recovery of its functional activity after warming.

The aim of this work was to develop an effective, available, and economic method for preservation of viability of human blood leukocytes at 0-2°C using an original PS.

MATERIALS AND METHODS

The study was carried out on 9 leukocyte concentrates prepared from the whole donor blood by cytapheresis on a Sorvell centrifuge with cooling (5 min at 2500*g*) in the presence of citroglucophosphate as the preserving agent. The mean content of the biological object in a Kompoplast-300 plastic container was 25±5 ml.

Before cooling, the biological material isolated in maximally viable state was exposed with PS (1:1). Three PS variants were used (PS-1, PS-2, PS-3) differing by the concentrations of the following ingredients (Table 1): mixed-effect cryoprotector (urea derivative) stabilizing fractions of intra- and extracellular water; restoring additive (succinic acid derivative) characterized by antioxidant, antihypoxic, membrane-stabilizing effects, which restores impaired structures and functions of cell membranes and inhibits LPO processes during storage; gelatinol (blood substitute); sodium citrate (anticoagulant); glucose, which, along with the energetic and plastic functions produces a slight protective effect. The names of the main ingredients are not presented, because application for registration of the invention has been submitted. The PS are not toxic; no washout of cell suspension after warming is needed.

After 20-min exposure the object in polypropylene tubes (1.5 ml) was placed into a refrigerator (0-2°C). In control series, the leukocytes were exposed under the same conditions without PS.

Experimental studies of the eosin resistance of leukocytes stored at 0-2°C for 8 days showed that the number of viable cells decreased significantly

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0007-4888/07/1443-0382

TABLE 1. PS Composition (Final Concentrations of Ingredients after Mixing with Leukocyte Suspension, %) for Leukocyte Storage at 0-2°C

Ingredient	PS-1	PS-2	PS-3
Cryoprotector of mixed action	7	10	13
Restoring additive	0.075	0.1	0.7
Glucose	0.35	0.35	0.35
Gelatinol	8	8	8
Sodium citrate	0.1	0.1	0.1

Note. Water *pro injection* was added into all PS to a volume of 200 ml.

TABLE 2. Morphofunctional Characteristics of Human Blood Leukocytes Subjected to 6-Day Hypothermia (0-2°C) with PS Variants ($M \pm m$)

Parameter, PS variant		Content		% of initial level
		before cooling	after warming	
Absolute cell count in 1 μl				
	Control	10 800±0	10 750±1909	99.50±17.68
	PS-	18350±0	8340±1045	99.72±12.57
	PS-2	17 140±7886	16 700±6575	88.83±6.79
	PS-3	28 500±0	27 400±3984	95.40±14.79
Granulocytes, %	Control	37±0	7±0	19±0
	PS-1	33±0	17.00±2.16	51.47±6.57+*
	PS-2	38.67±8.05	24.44±6.44	62.86±8.69+
	PS-3	24±0	8.75±2.21	36.36±9.27+*
Monocytes, %	Control	24±0	27.50±0.71	114.00±2.82
	PS-1	20±0	28.40±3.21	142.00±16.05+*
	PS-2	15.50±7.12	16.50±8.11	107.50±19.28
	PS-3	9±0	12.25±2.63	135.80±28.93
Lymphocytes, %	Control	41±0	65.50±0.71	159.00±1.41
	PS-1	48±0	59.40±6.35	117.20±13.22+
	PS-2	48.89±0.92	61.67±3.67	125.80±8.21+
	PS-3	63±0	80.75±4.99	128.00±80.16+
Eosin resistance, %	Control	99±0	66.00±15.56	66.00±15.56
	PS-1	100±0	84.80±3.63	84.80±3.63+
	PS-2	99.25±1.03	81.12±9.53	81.88±8.75
	PS-3	100±0	66.40±8.20	66.40±8.20*
Neutrophil phagocytic activity, %				
	Control	86±0	11.25±6.72	13.08±7.81
	PS-1	80±0	26.10±5.10	32.51±6.20+*
	PS-2	66.17±5.60	35.60±7.71	52.66±8.59+
	PS-3	45±0	16.50±0.70	37.00±1.40+*
LCP content in neutrophils, %				
	Control	2.32±0.00	1.53±0.66	65.50±28.00
	PS-1	2.54±0.00	2.20±0.30	86.70±10.81
	PS-2	2.53±0.03	2.46±0.22	96.89±7.93+
	PS-3	2.24±0.00	1.72±0.29	76.60±12.99*

Note. Number of observations for PS-1 and PS-3: 5; for PS-2: 9. $p<0.05$ compared to: +control, *PS-2.

(to 35.67±3.21% of initial level) by day 7. Therefore, subsequent experiments were carried out for a period of observation of 6 days.

The leukocytes were warmed at ambient temperature for 2-3 min in tubes (the tubes were gently shaked twice per sec).

The parameters of the initial and warmed suspension were evaluated: total and differential leukocyte count, leukocyte viability [6], phagocytic activity of neutrophils [6], and bactericidal activity of neutrophils (by the content of lysosomal cationic proteins (LCP) [7].

The data were processed statistically using paired Student's test [2].

RESULTS

The counts of eosin-resistant cells, granulocytes, LCP-containing neutrophils, and neutrophils capable of phagocytosis after warming of the biological object in control series (without PS) were significantly ($p<0.05$) lower than in experiments with leukocyte suspension exposed with PS (Table 2).

In experimental series, the percent of cells after 6-day storage with any of PS variants and warming of the leukocyte concentrate decreased by no more than 12-18%.

Analysis of morphological composition of leukocytes exposed to hypothermia (0-2°C) once more confirmed higher resistance of lymphocytes and monocytes to destructive factors and lower resistance of granulocytes, due to their more intricate cell organization [1]. The percentage of lymphocytes and monocytes in the leukoconcentrate increased after warming because of reduction of granulocyte count (Table 2). The results indicate that PS-2 significantly better ($p<0.05$) preserved morphological integrity of granulocytes than PS-1 and PS-3.

Evaluation of leukocyte viability with vital stain (eosin) [8] showed better preserving characteristics of PS-1 and PS-2. After exposure with these solutions, 84.80±3.63 and 81.80±8.75% cells (from initial content) retained intact membranes after 6 days, while after exposure with PS-3 this parameter was significantly ($p<0.05$) lower.

Phagocytic activity determines biological integrity of neurtophils. Its evaluation in the latex test [4] showed that after exposure and 6-day storage at 0-2°C with PS-1 and PS-3 and subsequent warming, only 32.51±6.20 and 44.00±12.17% neutrophils, respectively, retained the capacity to phagocytosis, which was significantly ($p<0.05$) lower than after exposure with PS-2 (Table 2).

The content of LCP is an indicator of bactericidal activity of neutrophils without O_2. After 6-day exposure with PS-2, the content of LCP was significantly ($p<0.05$) higher than in experiments with PS-3, while the results of PS-1 and PS-2 treatment were virtually the same. Analysis of the data showed that PS-2 exhibited the best protective characteristics by the majority of parameters.

This method for preserving human blood leukocytes is available, requires no expensive equipment and reagents, and can be used in practical medicine and biology.

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