Experimental Study of Corrective Effects of Human Umbilical Blood Nuclears under Conditions of a Posthemorrhagic State

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Intravenous transfusion of nucleated cells from human umbilical cord blood (10^6/ml saline) to female rats 3 h after massive blood loss produced a pronounced corrective effect (day 5): alleviates posthemorrhagic changes and normalizes hemoconcentration parameters, metabolic disorders, and behavioral reactions.

Key Words: *acute hemic hypoxia; posthemorrhagic status; human umbilical blood; nuclear cells; behavioral reactions*

The use of nucleated cells (NC) from human umbilical cord blood (UCB) for transplantation as an alternative variant for hemopoiesis deficiency compensation in severe diseases of the hemopoietic system, liver, kidneys, immune system, metabolic disorders, *etc*. attracts special interest of clinicians during the latest decade [1,3-5]. Experimental and clinical studies of Russian and foreign authors demonstrated high hemopoietic potential of UCB containing a great number of precursor cells. These cells include stem and progenitor cells giving rise to mature blood cells stem (CFU) and providing rapid recovery of the immune system in the recipients [6,10,11]. High therapeutic effect of human UCB cells was demonstrated on various experimental models [2,7,9]. However, many problems concerning the use of these cells in this or that disease, various mechanisms of their differentiation and functioning, periods and intensity of corrective effects after transfusion, *etc.*, remain unsolved.

Since hypoxia is the pathogenetic basis of many pathologies associated with dysfunctions of the respiratory and cardiovascular systems and the blood transporting system, we studied possible corrective effects of UCB NC in acute hemic hypoxia and the resultant posthemorrhagic status in rats.

MATERIALS AND METHODS

Experimental studies were carried out on 19 outbred female rats (235-250 g) in accordance with the Laboratory Practice Regulations in the Russian Federation, approved by the Order No. 267 of the Ministry of Health of the Russian Federation of June 19, 2003. In order to evaluate the corrective effects of umbilical blood nuclears, acute hemic hypoxia was induced by blood collection (30% circulating blood volume) from the sublingual veins in rats, which was followed by the development of posthemorrhagic status.

Nuclear cells were isolated from human umbilical blood by centrifugation in Ficoll-verograffin density gradient (ρ=1.007; 30 min, 500*g*). The interphase ring cells were collected and washed by centrifugation. Cell concentration was evaluated in a Goryaev chamber and adjusted to 10^6 cell/ml with saline. Cell phenotype corresponded to standard values for UCB (50-60% CD3, 30-40% CD4, 20-30% CD8, 15-20% CD19, 3-6% CD16).

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The animals were divided into 3 groups. Group 1 (n=6) animals were subjected to blood loss and received no therapy. Group 2 (n=7) rats were injected with concentrated suspension of umbilical blood nuclear cells after blood loss. Group 3 (n=6) were intact animals.

Group 2 rats were injected (into the tail vein) with umbilical blood nuclears (10^6 NC/ml saline) 3 h after blood loss. Hence, the NC dose was 4×10^6/kg, which corresponded to NC dose injected to humans for hemopoiesis substitution [10,11]. The animals were euthanatized on day 5 after acute hemic hypoxia.

The animals were examined before modeling of acute hemic hypoxia and euthanasia. Open field behavior and hole reflex characterizing the locomotor, emotional, and exploratory activities were studied. The horizontal (number of crossed squares) and vertical (rearing episodes) activities, grooming episodes, hole reflex (number of holes explored), and body weight gain were recorded. Peripheral blood parameters (hemoglobin content, leukocyte and erythrocyte counts) were evaluated and metabolic shifts were studied by plasma biochemistry (ALT, AST, creatinine, urea, and alkaline phosphatase, AP) on a Konelab-30i analyzer.

The results were statistically processed using Student's t test.

RESULTS

The findings indicated a trend to a lesser body weight gain in both experimental groups, because of the absence of weight gain or even its loss (by 5-8 g) in some animals, while in controls body weight increased by 9-10 g by day 5.

Open field testing showed a decrease in motor and emotional activities in group 1 rats after acute blood loss. This was seen from a lesser number of running, rearing, and grooming episodes in comparison with the control (Table 1). Exploratory activity also decreased (hole reflex was inhibited), which was characteristic of a hypoxic status.

Infusion of umbilical blood nuclears (group 2) partially or completely normalized these shifts; parameters of behavioral reactions approached those in control rats.

The coordination of behavioral reactions in the open field test was modified under the effect of blood loss. In controls, the increase in the number of running and rearing episodes correlated in 67% animals. In group 1, the percent of animals with these behavioral manifestations decreased 2-fold (33%), while in group 2 the parameters not only normalized, but the percent of animals (85%) with high activity increased 2.6 times in comparison with group 1 and 1.3 times in comparison with the control. Similar effect (significant improvement of behavioral reactions and motor activity) was observed after transplantation of human umbilical blood mononuclears to rats with experimental ischemic stroke [12].

Low hemoglobin level and low erythrocyte count in the peripheral blood impairing its oxygen transporting function are an important signs of anemic hypoxia. The groups of experimental animals did not differ by the initial status of blood concentration values. On day 5, hemoglobin level in experimental animals significantly decreased compared to the control (Table 2). In group 1, hemoglobin content decreased by 6.9±0.6 g/% in comparison with the basal level and erythrocyte count decreased by 1.3 times compared to basal level and by 1.4 times compared to the control, which indicated the development of the posthemorrhagic status.

In group 2, injection of UCB NC to females after blood loss decelerated the drop of hemoglobin level (by 3.6±0.2 g/%) in comparison with the basal level.

TABLE 1. Behavioral Reactions of Rats before and on Day 5 after Blood Loss and UCB NC Injection ($M\pm m$)

Parameter		Group		
		1	2	3
Number of running episodes	basal	38.0±6.9	29.0±4.0	35.3±7.6
	experiment	24.6±2.6*	42.3±4.1+	37.6±4.3
Number of rearing episodes	basal	4.0±1.9	1.6±0.4	4.0±1.2
	experiment	3.0±1.1*	4.4±1.3	6.6±1.1
Grooming	basal	3.4±0.9	4.3±1.5	6.5±2.4
	experiment	2.6±0.8*	3.8±0.4	5.8±0.9
Hole reflex	basal	4.6±1.3	4.0±0.9	4.5±1.2
	experiment	3.8±1.2*	8.0±1.4+	7.7±1.1

Note. Here and in Tables 2, 3: $p<0.05$ compared to: *control, +group 1.

TABLE 2. Morphological Composition of Peripheral Blood of Rats on Day 5 after Blood Loss and UCB NC Injection (*M*±*m*)

Group	Hemoglobin, g/%	Leukocytes, cell/ml	Erythrocytes, mm^3
1	13.0±0.7*	(9.6±0.8)×10^6	(510.8±28.2)×10^{4*}
2	15.9±0.4*$^+$	(10.2±0.6)×10^6	(645.4±22.8)×10^{4+}
3	18.9±0.4	(10.1±0.6)×10^6	(694.0±26.8)×10^4

TABLE 3. Plasma Biochemistry in Rats on Day 5 after Blood Loss and UCB NC Injection (*M*±*m*)

Parameter	Group		
	1	2	3
ALT, U/liter	72.6±3.8	64.7±2.9	68.3±3.6
AST, U/liter	302.3±16.9*	229.2±11.1$^+$	228.8±14.5
Creatinine, μmol/liter	40.6±1.0	40.0±0.7	41.4±0.6
Urea, mmol/liter	5.8±0.3	6.1±0.2	5.8±0.3
AP, U/liter	699.3±16.1*	637.4±15.8$^+$	619.5±11.9

Erythrocyte count decreased by 1.1 times, which was significantly higher than in group 1 and close to the control level. Peripheral blood leukocyte count in group 2 was somewhat higher than in group 1, but did not differ from the control. These data on the one hand can indicate the presence and circulation of injected cells and on the other reflect the so-called cytokine stimulation of hemopoiesis or summation of these effects.

Important pathogenetic components of hypoxia, both acute and chronic, are hemodynamic and metabolic disorders, developing as a result of oxygen starvation. Biochemical analysis of the plasma of rats, subjected to blood loss, found elevated AST and AP activities in comparison with groups 2 and 3. This indicated disorders of liver function and hypoxia development (Table 3). Other enzymes did not change. Virtually no metabolic changes were found in group 2, this presumably indicating a protective effect of UCB NC, preventing the formation of the "vicious circle" of metabolic changes, triggered by acute circulatory and hemic hypoxia.

Hence, the results of our experiments indicate that infusions of UCB NC to animals after acute massive blood loss produces a sufficient antihypoxic effect in female rats and reduces the severity of posthemorrhagic changes. The drop in hemoglobin level was less pronounced, erythrocyte counts and biochemical values approached the normal, and shifts in behavioral reactions of rats were corrected. This effect after blood loss can be regarded as positive, indicating the development of a pronounced compensatory reaction (hemopoietic tissue regeneration and elimination of the neurological deficiency).

The mechanism underlying the effect of UCB NC will be the object of further studies. It is important to clear out whether the increase in erythrocyte count in the blood of rats is a result of colony-forming activity of blood cells (erythrocyte precursors) or the injected concentrated suspension is characterized by a peculiar nonspecific effect, mediated by the cytokine effects on the bone marrow or other organs of animals.

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