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INFLUENCE OF OXYGENATOR EXTRACORPORAL CIRCUIT TREATMENT WITH ADAPTATION COMPOSITION (AdC) ON MORPHOLOGICAL CHANGES OF ERYTHROCYTES

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Summary

Background. The study highlights a method of treatment extracorporeal circuit with «adaptation composition» (AdC) for the reduction of negative impact on state of erythrocytes.

Aim: to assess the effect of treatment the contact surface extracorporeal circuit with «adaptation composition» (AdC) on changes in the state of erythrocytes after open-heart surgery with cardiopulmonary bypass (CPB).

Material and methods. A total of 90 patients were enrolled, they were divided into two groups. The group 1 (45 patients, 39/6 male/female) included patients who underwent surgical procedures without treatment of an extracorporeal circuit with AdC. The group 2 (45 patients, 39/6 male/female) included patients who underwent surgery with the treatment of an extracorporeal circuit with AdC. According to the study protocol, patient blood was sampling for complete blood cell count (CBC) and erythrocyte morphology at 4 stages of surgery: before surgery, at 10 min. CPB-time, at 60 min. CPB-time and after separation from CPB.

Results. The albumin of AdC creates a protective nanolayer on the surface of the oxygenator membrane and tubes. There were no statistically significant differences of parameters in the groups before CPB. Level of Ht 2 (group 2) at 60 min CPB-time and after CPB, were lower than Ht 1 (group 1) ($p=0.021$ and $p=0.035$ correspondingly) because MCV1 was higher ($p=0.025$ and $p<0.0001$ correspondingly). The increase MCHC in groups at 10 min. CPB-time relatives with the decrease in MCV at 10 min CPB-time. At 60 min, there are changes of RDWa2 76.05 ± 5.46 and RDWa1 72.35 ± 7.26 , $p<0.000$. After CPB higher content of reticulocytes ($p < 0.0001$), echinocytes ($p < 0.0001$) and spherocytes ($p < 0.0001$) is observed in group 1. The lowering of mechanical resistance ($p = 0.04$) and increasing membrane permeability for urea were in group 1. After CPB the best acid hemolysis resistance was in group 2 ($p = 0.05$), erythrocytes were more resistant to hypoosmotic factor ($p = 0.01$) in group 2.

Conclusion. The treatment of oxygenator with AdC reduces the negative influence CPB on state of RBC. Membranes of erythrocytes were more resistant to traumatic factors in the group with AdC.

Key words: cardiopulmonary bypass, oxygenator, erythrocyte morphology, adaptation composition.

INTRODUCTION

A special biological function is performed by the erythrocyte membrane as a universal model that reflects the state of the membranes of the whole organism. Cell membranes are the first target when are influenced by hypoxia, their changes can serve as an early signal of the pathological process [1]. During heart surgery, the problem of hypoxia and its prevention remains a priority. Membranes of the oxygenator are the active part of the device, where blood oxygenation is taken place. Unfortunately, for the

patient's organism, its surface is an exogenous material that leads to the adsorption of erythrocytes and the adhesion of leukocytes, which in turn provokes an inflammatory response [2-6]. All cells of the blood are sensitive to these factors and can be irreversibly damaged.

On the other hand, in our previous study [7] it was shown that the adsorption of erythrocytes leads to a decrease in cell metabolism on the membrane of the oxygenator and a decrease in the number of the oxygenated erythrocytes was associated with it. This phenomenon is

accompanied by changes in the shape and state of red blood cells. Changes in the shape of erythrocytes can give some information about the intensity of the pathological process [8]. In normal human blood, the discocytes (erythrocytes of biconcave shape) are the main bulk (80-90%). In addition, there are planocytes (with a flat surface) and aging forms of erythrocytes:

- spiny erythrocytes or echinocytes (about 6%)
- domed erythrocytes or stomatocytes (about 1-3%)
- spherical erythrocytes or spherocytes (about 1%).

The process of changing the shape of an erythrocyte is its transformation from a disk to a spherical shape. This process is carried out in two ways: 1) echinocytosis when the surface of the erythrocyte is covered with cone-shaped spines. This occurs under the action of fatty acids, ATP deficiency, increased pH. Echinocytes are often formed when suspending erythrocytes in an isotonic environment and the addition of albumin can return the cells to a normal discocyte form and 2) stomatocytosis when the erythrocyte retains a smooth surface but takes the shape of a unilaterally concave disc and one has an increased volume and area of 20-30%. This occurs when the pH is lowered and under the influence of drugs. Echinocyte and stomatocyte are the reverse forms of erythrocyte, and spherocyte is the most rigid structure of erythrocyte, which is irreversible and precedes cell destruction [8]. Cell death occurs either through necrosis and apoptosis. These are opposite forms both in regard to the reasons causing them and to the mechanism of development. A sign of cell necrosis is more often its swelling (necrotic increase in cell volume), and a decrease in cell volume prevails in apoptosis (apoptotic decrease in cell volume). Echinocytes are preapoptotic cells and stomatocytes are pre-necrotic [16].

The resistance of erythrocytes to various influences of internal and external environments is also caused by a condition of its cell membrane. Normal permeability of the cytomembrane is the main requirement in cell homeostasis. Decreased resistance of erythrocytes in hypoxia leads to increased hemolysis. Previously, hemolysis in the process of CPB was considered only as mechanical damage of erythrocytes as a result of cardiotomy suction power, roller pump capacity, the presence of an arterial filter and turbulent flows in places of transitional diameters of the extracorporeal circuit. The measurement of plasma free hemoglobin (plfHb) concentration to characterize hemolysis is a routine method but sublethal trauma of RBC is more difficult to detect because no simple direct test exists. The concept of sublethal RBC damage was introduced by Dr. Galletti [17, 18]. The study of erythrocyte membrane resistance allows us to judge the resistance of erythrocytes to deformation and RBC mechanical fragility within the conditions of CPB [9]. One of the main goals to reduce intraoperative hemolysis was to develop the most biocompatible components of the extracorporeal circuit. In this regard, it is important to simultaneously conduct a comprehensive assessment of the resistance of erythrocytes to various hemolytic factors

and the permeability of their plasma membrane. In clinical practice, there is a need for an available assessment method of the functional status of the erythrocyte's membrane.

An appropriate way to assess the structural and functional state of erythrocytes is to determine the resistance of blood cells to various factors – mechanical, osmotic and acidic. We extrapolate to the hemolysis time of red cells.

By contrast with most other cells, erythrocytes placed in an acidic medium undergo a high-grade hemolytic reaction (necrosis). It is known that the acid resistance of erythrocytes mainly reflects the state of the phospholipid bilayer and erythrocyte membrane proteins. The main target of acid hemolysis is membrane proteins and cell destruction is preceded by the effect of hemolysin on structural elements of a membrane and the transport of this lysing agent into the cell, which may be the result of harmful effects of the agent and pore formation in the lipid bilayer [10].

Osmotic resistance of erythrocytes (ORE) generally characterizes the state of the cytoskeleton of cells. Osmotic hemolysis occurs when a cell enters a hypotonic environment. Hypotonic solutions lead to cell swelling and lysis if the osmotic movement of water is great enough.

Urea molecules upon the concentration gradient penetrate through the pores into the cell, capturing water molecules, creating there an increased osmotic pressure, which leads to its lysis. Membrane pores have considerably better permeability to small water molecules than to larger urea molecules, therefore the erythrocyte membrane permeability (EMP) test for urea is more sensitive [9].

The purpose of the study was to assess the effect of treatment the contact surface extracorporeal circuit with «adaptation composition» (AdC) on changes in the state of erythrocytes after open-heart surgery with cardiopulmonary bypass (CPB).

MATERIALS AND METHOD

On the basis of the surgical center of SIS «RPC PCM» SAD adult patients were operated with CPB by the cardiac surgery team. A total of 90 patients were enrolled. This study complied with the ethic committee approval and written informed consent was obtained from patients. Patients were divided into two groups. The group 1 (45 patients, 39/6 male/female correspondingly) included patients who underwent surgery without treatment of extracorporeal circuit by adaptation composition. Complete analysis results of group 1 before, during and after CPB is shown in tab.1. The group 2 (45 patients, 39/6 male/female correspondingly) included patients who underwent surgery with the treatment of an extracorporeal circuit with adaptation composition. Complete analysis results of group 2 before, during and after CPB is shown in tab.2 The age of patients in both groups ranged from 68 ± 13 years. The weight of patients ranged from 55 to 115 kg, with an average

of 86.4 ± 5.85 kg. According to the classification system of the New York Heart Association (NYHA), patients were distributed: III FC $n = 75$ (68.2%), II FC $n = 35$ (31.8%). Coronary artery bypass grafting (CABG) was performed with ventricular fibrillation, $n = 89$ (80.9%), the rest of the surgeries (cardiac valve surgeries), $n = 21$ (19.1%), performed using cardioplegic solution «Custodiol». The mean CPB time was 95.65 ± 12.18 min. In the group with «Custodiol» mean aortic cross-clamp time was 74.3 ± 12.5 min. The perfusion system used a membrane oxygenator, roller pump, nonpulsatile flow and the primed circuit 1.3-1.6 l. to achieve moderate hemodilution ($Ht - 25 \pm 2$ г/л).

Hyperosmolar prime volume with an osmolarity of 510.9 mosmol/l was used [11]. The AdC was prepared according to the proposed methodology [12]. The mean blood flow and mean arterial blood pressure were targeted at 2.5 L /min/m² and 60-80 mmHg, correspondingly. CPB was administrated in conditions with moderate systemic hypothermia (32-33°C).

The oxygenator was treated with AdC according to the pattern: before surgery, 20 ml of subclavian vein patient blood was collected. The blood was sedimentation for 15 min. to form a clot, then to obtain the serum it was centrifuged using a centrifuge NF 200 (Nuve, Turkey) for 7 minutes at 3500 rpm. The obtained serum of 5-10 ml was taken with a sterile syringe, adjusted to a volume of 20 ml with saline (0.9% NaCl) and add into the oxygenator (pre-filled with 0.9% NaCl in a volume of 1000 ml) through a disposable membrane filter with pores 0.22 μ m (Minisart, Sartoriusstedium, Biotech corp.) Subsequently, the AdC was circulated at idle operating mode for 5-7 minutes. Then the content of the oxygenator was completely drained and then carried out the standard procedure of filling the oxygenator with corresponding solutions.

According to the study protocol, patient blood was sampling for complete blood cell count (CBC) and cytological analysis at 4 stages of surgery: before surgery, at 10 min. CPB-time, at 60 min. CPB-time (rewarming stage) and after separation from CPB.

CBC was carried out on the hemolytic analyzer of Swelab Alfa Basic (Sweden) which allows us to estimate the size of erythrocytes.

The electrophoretic analysis of the composition of AdC was accomplished before and after the treatment of an oxygenator at the Institute of Biochemistry. O. V. Palladin NAS of Ukraine in the department of protein structure.

Plasma free hemoglobin (plfHb) concentration was measured using the hemoglobincyanide method.

Erythrocytes osmotic resistance was carried out by the method of Gottfried and Robertson to determine the time up to 50% hemolysis of a blood sample in a buffer hypotonic glycerol-saline mixture in one tube.

Mechanical resistance of erythrocytes. The method of Y. V. Ganitkevich, L. I. Chernenko in its own modifi-

cation was applied to determine mechanical resistance of erythrocytes, recording the concentration of plasma free hemoglobin in a 2% suspension of erythrocytes (diluent – Ringer's solution, buffered HEPES [pH = 7.4] and containing 0,1% glucose), subjected to mechanical impact, by centrifugation for 30 min at 1500 rpm. The result was expressed as% of hemolyzed cells after mechanical exposure, taking for 100% hemolysis the content of plfHb in 2% hemolysate of erythrocytes (diluent was distilled water).

Erythrocyte membrane permeability (EMP) for low molecular weight hydrophilic substances was determined using the method of urea hemolysis [13]. The method involves increasing the concentration of urea (osmotically active and does not penetrate through the intact membrane) in a series of buffered hypotonic solutions. We studied the degree of increase in hemolysis depending on the concentration of urea and thus judged the degree of damage to the erythrocyte membrane.

Acid hemolysis. The essence of the method is that 2 ml of 0.004 M HCl solution is added in a cuvette with 20 mm³ of blood and 2 ml of saline and than the time at which 100% hemolysis of erythrocytes occurs is calculated.

Statistical analysis. MedStart software program was used for the statistical analyses. We checked for normality of data before further analysis; p-values were calculated for continuous variables using the Student's test. Group differences were considered statistically significant with a p-value of < 0.05.

RESULTS AND DISCUSSION

Electrophoresis. Separation of the proteins that are a part of the AdC before and after oxygenator treatment is shown in Fig. 1, where changes in albumin concentration in the original AdC solution and after circulation at idle operating mode for 5 minutes are clearly observed. So, on the path № 1, the molecular mass is marked; path № 2 and 4 correspond to AdC at a dilution of 1: 200, that was added to the oxygenator; path № 3 and 5 correspond to AdC after 5 minutes of circulation in an extracorporeal circuit at idle operating mode. The albumin impoverishment is the indication of its utilization to create a nanolayer coating on the surface extracorporeal circuit and there is sufficient quantities of residual albumin in the AdC to produce the nanolayer.

Cytological research. These researches specify that the use of CPB influences of erythrocytes and indicate that there are changes in the shape and size of erythrocytes under the affect of CPB (fig. 2B, 3B).

In group 1, the microscopy of blood smear detects the presence of a significant number of microcytes, spherocytes and echinocytes after CPB. (fig. 2B). The presence of irregularly shaped erythrocytes and the phenomenon of erythrocyte agglutination is also detected, which is additional evidence of their defective state. The change in the shape of erythrocytes from a «biconcave lens» to a spherocyte indicates a decrease in the effective surface area of erythrocytes (1.7 times).

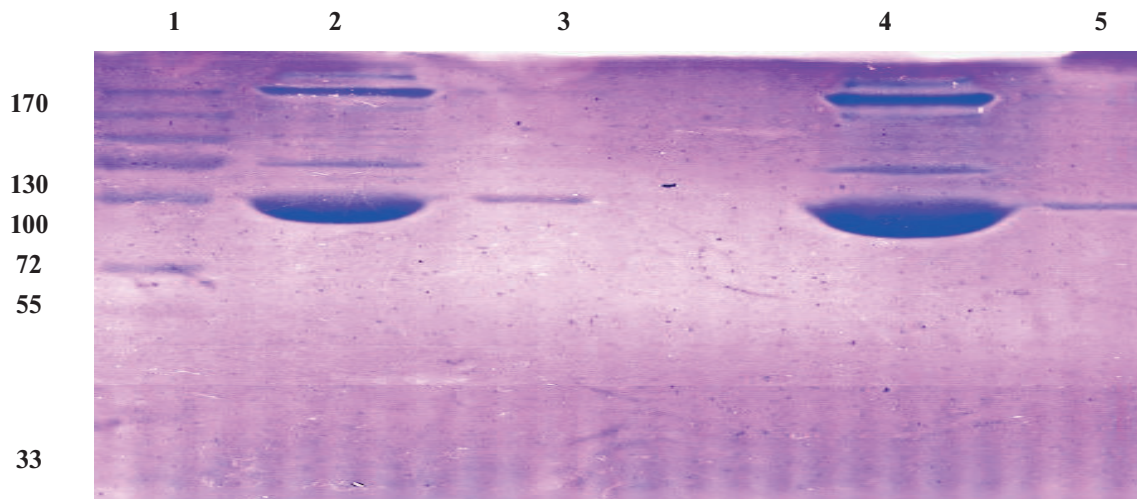
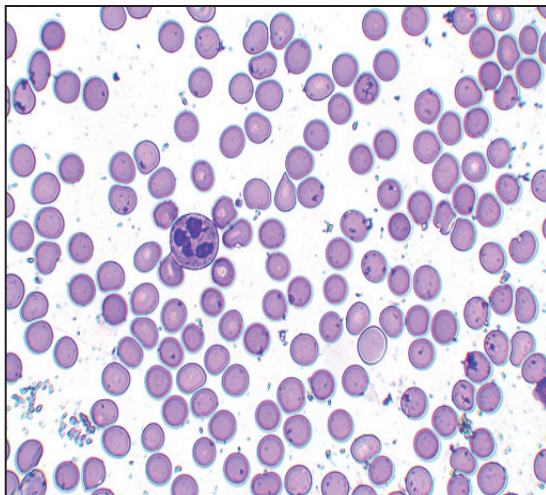
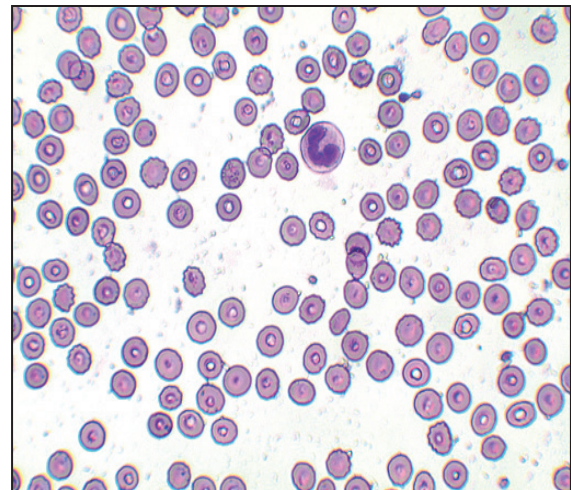


Fig. 1 Electrophoregram of separation blood proteins of original AdC and after circulation in an extracorporeal circuit at idle operating mode. 1- molecular mass marker; 2 and 4 the original AdC at a dilution of 1: 200; 3 and 5 – the AdC is later 5 minutes of circulation at idle operating mode.

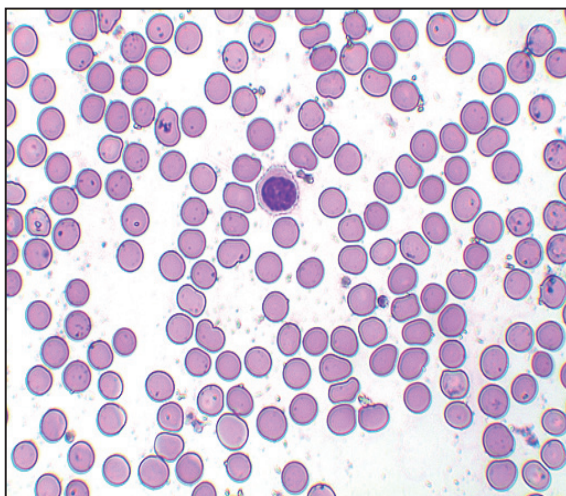


A – before CPB

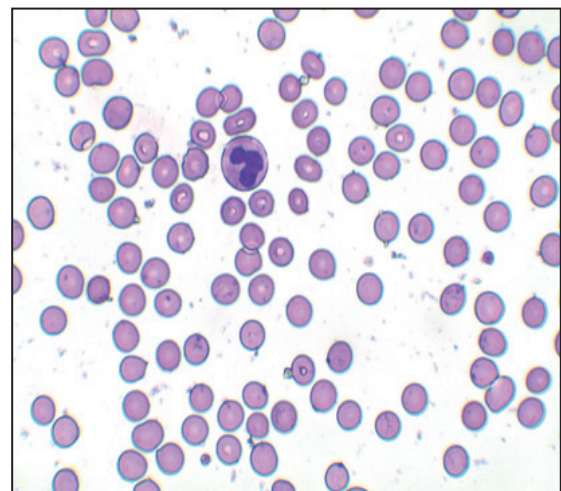


Б – after CPB

Fig.2 Distinctive blood smear for group 1 at different stages of surgery.



A – before CPB



Б – after CPB

Fig.3 Distinctive blood smear for group 2 at different stages of surgery.

It can indicate the presence of certain tissue hypoxia. The loss of some erythrocytes on an oxygenator membrane and tubes due to their irreversible adsorption and restriction of access to the gas exchange membrane of cells leads to a decrease in oxygen supply to tissues.

The cytological research of blood samples in group 2 (with use of AdC) before and after CPB (Fig. 3 A, B) indicates more preservation of erythrocytes of their shape, and only individual cells change their shape.

Tab.1

Parameters of red blood cells at different stages of surgery. Group 1 (M ± m)

Parameter	Before CPB	10 min. CPB-time	p	60 min. CPB-time (rewarming)	p*	After CPB	p**	p***
RBC	4.57 ± 0.43	3.08 ± 0.31	< 0.0001	2.94 ± 0.42	0.096	3.69 ± 0.32	< 0.0001	< 0.0001
Hb	133.18 ± 11.31	83.14 ± 19.02	< 0.0001	85.33 ± 9.98	0.360	109.25 ± 22.69	0.0002	< 0.0001
Ht	40.44 ± 4.01	24.49 ± 3.32	< 0.0001	25.44 ± 3.9	0.073	33.01 ± 1.70	< 0.0001	< 0.0001
Hemolysis	0.16 ± 0.08	0.18 ± 0.08	0.126	0.39 ± 0.17	< 0.0001	0.51 ± 0.19	< 0.0001	< 0.0001
MCV	86.78 ± 3.49	82.34 ± 4.93	0.0068	84.88 ± 4.37	0.01	88.29 ± 4.25	< 0.0001	0.002
MCH	28.76 ± 1.32	29.50 ± 2.71	0.121	28.76 ± 1.77	0.400	29.48 ± 1.17	0.061	0.450
MCHC	332.57 ± 14.22	348.61 ± 23.21	0.008	330.38 ± 16.38	0.611	325.14 ± 21.16	0.013	< 0.0001
RDW%	13.08 ± 0.83	13.11 ± 1.03	0.559	12.46 ± 0.70	0.025	13.75 ± 1.09	< 0.0001	0.001
RDWa	74.91 ± 7.32	74.40 ± 6.80	0.134	72.35 ± 7.26	0.010	77.56 ± 7.13	< 0.0001	< 0.0001

p – significance level in comparison parameters before CPB and at 10 min. CPB-time; p* – significance level in comparison parameters at 10 min. CPB-time and 60 min. CPB-time (rewarming); p** – significance level in comparison parameters at 60 min. CPB-time (rewarming) and after CPB; p*** – significance level in comparison parameters before CPB and after CPB.

Tab.2

Parameters of red blood cell at different stages of surgery. Group 2 (M ± m)

Parameter	Before CPB	10 min. CPB-time	p	60 min. CPB-time (rewarming)	p*	After CPB	p**	p***
RBC	4.63 ± 0.54	3.04 ± 0.50	< 0.0001	3.13 ± 0.72	0.148	3.74 ± 0.45	0.002	< 0.0001
Hb	130.38 ± 12.91	84.41 ± 15.35	< 0.0001	89.85 ± 14.72	0.096	105.65 ± 29.57	< 0.0001	< 0.0001
Ht	39.54 ± 4.11	24.92 ± 4.55	< 0.0001	26.05 ± 4.26	0.161	32.45 ± 4.89	< 0.0001	< 0.0001
Hemolysis	0.13 ± 0.06	0.15 ± 0.06	0.170	0.33 ± 0.13	< 0.0001	0.42 ± 0.11	0.0002	< 0.0001
MCV	85.40 ± 3.81	81.19 ± 4.00	< 0.0001	82.32 ± 3.40	0.174	86.79 ± 2.54	< 0.0001	0.331
MCH	29.39 ± 1.24	28.68 ± 1.56	0.288	29.89 ± 1.72	0.334	29.10 ± 1.59	0.088	0.658
MCHC	333.52 ± 10.32	342.07 ± 11.32	0.013	338.23 ± 12.33	0.215	332.14 ± 12.49	0.038	0.189
RDW%	13.30 ± 0.90	13.09 ± 0.95	0.471	13.37 ± 0.93	0.789	13.57 ± 0.85	0.478	0.816
RDWa	77.61 ± 5.84	76.10 ± 4.83	0.189	76.05 ± 5.46	0.400	75.66 ± 5.12	0.709	0.256

p – significance level in comparison parameters before CPB and at 10 min. CPB-time; p* – significance level in comparison parameters at 10 min. CPB-time and 60 min. CPB-time (rewarming); p** – significance level in comparison parameters at 60 min. CPB-time (rewarming) and after CPB; p*** – significance level in comparison parameters before CPB and after CPB.

The complete blood cells. A comparison of the studied results of hematological indexes, showed that at 10 min. cardiopulmonary bypass time (CPB-time) there is a statistically significant decrease in Hb, Ht and erythrocytes both group 1 and group 2 (p < 0.0001) due to hemodilution. After surgery in 1 and 2 groups the in-

crease in Hb is noted, in comparing two groups, parameters were statistically not significant (p=0,907) in spite of the fact that in 1 group hemolysis was higher (p < 0.0001). Possibly it is because of more peripheralization of reticulocytes in group 1 compared with group 2 (p < 0.0001).

Tab.3

Comparisons of parameters of a red blood cell and dynamics of change of erythrocytes shape between group 1 and group 2 (M ± m)

Parametr	Before CPB Group 2	Before CPB Group 1	<i>p</i>	10 min. CPB-time Group 2	10 min. CPB-time Group 1	<i>p</i> *	60 min. CPB-time (rewarming) Group 2	60 min. CPB-time (rewarming) Group 1	<i>p</i> **	After CPB Group 2	After CPB Group 1	<i>p</i> ***
RBC	4.63 ± 0.54	4.57 ± 0.43	0.071	3.04 ± 0.50	3.08 ± 0.31	0.532	3.13 ± 0.72	2.94 ± 0.42	0.051	3.74 ± 0.45	3.69 ± 0.32	0.648
Hb	130.38 ± 12.91	133.18 ± 11.31	0.334	84.41 ± 15.35	83.14 ± 19.02	0.591	89.85 ± 14.72	85.33 ± 9.98	0.062	105.65 ± 29.57	109.25 ± 22.69	0.907
Ht	39.54 ± 4.11	40.44 ± 4.01	0.418	24.92 ± 4.55	24.49 ± 3.32	0.659	26.05 ± 4.26	25.44 ± 3.90	0.021	32.45 ± 4.89	33.01 ± 1.70	0.035
Hemolysis	0.13 ± 0.06	0.16 ± 0.08	0.061	0.15 ± 0.06	0.18 ± 0.08	0.092	0.33 ± 0.13	0.39 ± 0.17	<0.0001	0.42 ± 0.11	0.51 ± 0.19	<0.0001
MCV	85.40 ± 3.81	86.78 ± 3.49	0.381	81.19 ± 4.00	82.34 ± 4.93	0.368	82.32 ± 3.40	84.88 ± 4.37	0.025	86.79 ± 2.54	88.29 ± 4.25	<0.0001
MCH	29.39 ± 1.24	28.76 ± 1.32	0.113	28.68 ± 1.56	29.50 ± 2.71	0.487	29.89 ± 1.72	28.76 ± 1.77	0.780	29.10 ± 1.59	29.48 ± 1.17	0.114
MCHC	333.52 ± 10.32	332.57 ± 14.22	0.137	342.07 ± 11.32	348.61 ± 23.21	0.149	338.23 ± 12.33	330.38 ± 16.38	0.925	332.14 ± 12.49	325.14 ± 21.16	<0.0001
RDW%	13.30 ± 0.90	13.08 ± 0.83	0.421	13.09 ± 0.95	13.11 ± 1.03	0.560	13.37 ± 0.93	12.46 ± 0.70	<0.0001	13.57 ± 0.85	13.75 ± 1.09	0.323
RDW a	77.61 ± 5.84	74.91 ± 7.32	0.08	76.10 ± 4.83	74.40 ± 6.80	0.078	76.05 ± 5.46	72.35 ± 7.26	<0.0001	75.66 ± 5.12	77.56 ± 7.13	0.021
Reticulocytes, %	1.41 ± 0.64	1.37 ± 0.60	0.762	-	-	-	-	-	-	3.51 ± 1.19	5.19 ± 1.46	<0.0001
Echinocytes, %	5.32 ± 0.79	5.10 ± 0.79	0.200	-	-	-	-	-	-	7.16 ± 0.84	9.79 ± 1.89	<0.0001
Spherocytes, %	0.73 ± 0.30	0.72 ± 0.32	0.894	-	-	-	-	-	-	2.19 ± 0.74	2.66 ± 1.17	<0.0001

p – significance level in comparison parameters before CPB and at 10 min. CPB-time; *p**- significance level in comparison parameters at 10 min. CPB-time and 60 min. CPB-time (rewarming); *p*** - significance level in comparison parameters at 60 min. CPB-time (rewarming) and after CPB; *p**** – significance level in comparison parameters before CPB and after CPB.

Level of Ht 1 (group 1) and Ht 2 (in group 2) being compared at 60 min CPB-time and after CPB, it is shown that Ht 2 was statistically lower than Ht 1 at these stages (*p*=0.021 and *p*=0.035 respectively). Simultaneously, at 60 min CPB-time and after CPB there was no statistical differences between Hb1 and Hb 2 (*p*=0.062 and *p*=0.907 respectively). We suggest that it is possible by significantly increasing the MCV 1 compared to MCV 2 (group 2) at these stages (*p*=0.025 and *p*<0.0001 respectively).

Decline of mean corpuscular volume (MCV) at 10 minutes CPB-time as in group 1 (MCV 1) 82.34 ± 4.93; *p*=0.0068 and in group 2 (MCV 2) 81.19 ± 4.00; *p*<0.0001 attracts attention. It is possible due to the use of the hyperosmolar primary solution, and this MCV remain within the physiologically normal state. There is no significant difference in MCV at 10 min. CPB-time between group 1 and group 2 (*p*=0.381). It demonstrates that the prime composition of equal influences of erythrocytes in two groups. There are differences *p*=0.025 between MCV 1 and MCV 2 at 60 min. CPB-time (rewarming) and *p*<0.0001 between MCV 1 and MCV 2 after CPB. Also there are

an increase in the mean corpuscular hemoglobin concentration (MCHC2) (*p*=0.013) in group 2 and in group 1 (MCHC1) (*p*=0.008) at 10 min. CPB-time. Possibly, this relative increase in parameters due to decrease MCV at 10 min CPB-time in group 1 (*p*=0.0068) and group 2 (*p*<0.0001). Again, the two groups being compared with each other and changes in MCV, MCH, MCHC at 10 minutes are statistically insignificant (tab. 3) that is indicative of an equal change in parameters from the beginning CPB in the two groups. MCHC1 being compared at 60 min. CPB-time and after CPB, there is a difference *p* = 0.013. There is a significance difference between MCHC1 before CPB and MCHC1 after perfusion (*p* < 0.0001). MCHC1 and MCHC2 being compared after CPB, causes there a significant decrease in the MCHC1 (*p*<0.0001).

Studying parameters of MCV after CPB by comparison before initiation of CPB suggested that an increase MCV was caused in group 1 (*p* =0.002) but this value does not differ (*p* = 0.331) in group 2 and decrease in MCV at 10 min. CPB-time (81.19 ± 4.00) returns to the basal value.

At 10 min. CPB-time in group 1 the red blood cell distribution width parameter (RDWa1) was caused 74.40 ± 6.80 and there was no significant difference between average values in comparison with basal value ($p=0.134$). The same situation was in group 2. RDWa2 was 76.10 ± 4.83 ($p=0.189$). Group 1 and group 2 being compared at 10 min. CPB-time, there was no significant difference between average values ($p=0.078$) but there were significant differences between RDWa 1 and RDWa 2 at 60 min. CPB-time ($p<0.0001$) and RDWa 1 and RDWa 2 after CPB ($p=0.021$).

At 60 min. CPB-time (rewarming stage), when comparing there is a change of RDW% 13.37 ± 0.93 (group 2) and 12.46 ± 0.70 (group 1); $p < 0.0001$ and RDWa 76.05 ± 5.46 (group 2), 72.35 ± 7.26 (group 1); $p < 0.0001$, at the same time in group 1 the tendency to decrease in RDWa is observed up to 60 min CPB-time ($p=0.010$),

it is possible due to more destruction macro – and microcytes on oxygenator membranes. This is consistent with higher hemolysis in group 1 compared with group 2 ($p < 0.0001$) at 60 min. In group 1 there are a statistically significant increase in RDWa1 after CPB in comparison with 60 min CPB-time ($p < 0.0001$). Probably this is due to an increase in the pool of reticulocytes in the peripheral blood (5.19 ± 1.46), as a reaction to more pronounced hemolysis and tissue hypoxia. CPB-time. There are significant differences $p < 0.0001$ between RDWa 1 before and after CPB.

In the study of the morphology of erythrocytes, it was found that the initial% content of reticulocytes, echinocytes and spherocytes of the two groups was not statistically significant (Tab. 3). After CPB in group 1 the higher content of reticulocytes ($p < 0.0001$), echinocytes ($p < 0.0001$) and spherocytes ($p < 0.0001$) is observed in the peripheral blood in comparison with group 2.

Tab. 4

Parameters of erythrocyte resistance before and after CPB ($M \pm m$)

Parameter		Group 1	Group 2	p
Mechanical resistance of erythrocytes, %	Before surgery	59.90 ± 18.21	45.28 ± 20.48	0.234
	After surgery	89.93 ± 19.87	61.38 ± 16.33	0.040
Time of acid hemolysis 50% of erythrocytes, sec.	Before surgery	230.39 ± 36.91	243.35 ± 38.92	0.104
	After surgery	134.48 ± 35.3	146.37 ± 22.82	0,05
Osmotic resistance of erythrocytes, sec.	Before surgery	448.2 ± 218.4	520.7 ± 319.05	0.206
	After surgery	263.9 ± 140.41	345.1 ± 173.56	0.01

p – significance level in comparison parameters before CPB and after CPB.

Tab. 5

Parameters of erythrocyte membrane permeability (TEM) for urea solution before and after CPB ($M \pm m$)

№ tube	TEM%, before CPB			TEM%, after CPB		
	Group 1	Group 2	p	Group 1	Group 2	p*
1 (40:60) *	43.87 ± 4.32	42.91 ± 3.18	0.583	42.44 ± 3.84	43.46 ± 4.53	0.749
2 (45:55)	47.45 ± 5.26	46.09 ± 6.07	0.656	46.82 ± 4.75	47.74 ± 3.91	0.818
3 (50:50)	73.10 ± 6.15	60.38 ± 7.98	0.097	69.44 ± 9.84	65.85 ± 8.88	0.05
4 (55:45)	86.43 ± 6.97	79.17 ± 8.32	0.076	81.78 ± 7.18	78.58 ± 9.66	0.041
5 (60:40)	91.43 ± 4.35	87.23 ± 5.06	0.209	90.47 ± 5.59	86.79 ± 4.72	0.003
6 (65:35)	95.08 ± 2.24	88.99 ± 3.87	0.077	95.76 ± 3.64	94.89 ± 3.89	0.343
7 (etalon)**	1.0000	1.0000		1.0000	1.0000	

* The ratio of hypotonic solutions of urea and sodium chloride.

** Etalon is the content of the tube is 100% urea solution.

p – significance level before CPB.

p* – significance level after CPB.

Hemolysis during extracorporeal circulation is the result of the destruction of the RBC membrane with breakdown and release of plasma free hemoglobin. There are no significant differences in the level of hemolysis between the basal value of hemolysis and at 10 min. CPB-time in groups, beginning with 60 min. CPB-time and after CPB hemolysis was higher in group 1 ($p < 0.0001$).

The study of erythrocyte resistance in both groups the following was revealed. Parameters of mechanical resistance of red blood cells before surgery were not statistically significant between the two groups ($p = 0.234$). In group 1 after separation from CPB, there was a decrease in the mechanical resistance of erythrocytes compared with group 2 ($p = 0.04$).

The analysis of erythrocyte membrane permeability for urea solution revealed that the level of 50% erythrocytes hemolysis in urea solution after CPB starting from dilution of hypotonic solutions of urea and sodium chloride in a ratio of 50:50 was higher in group 1, the same tendency remained in dilution 55:45, 60:40 (tab. 5). It may be explained both more damage of erythrocyte membranes in the extracorporeal circuit and more release of reticulocytes (young cell forms), which contain less cholesterol, which determines the density of location lipids in the membrane, viscosity and permeability of the lipid layer.

Assessment of acid erythrocyte resistance in group 1 demonstrates the reduction in acid erythrocyte hemolysis time of 50% after surgery compared to initial parameters ($p < 0.0001$), this may be due to the lytic action of the membrane-attacking complex when the complement system is activated under conditions of blood contact with a foreign surface extracorporeal circuit. After weaning from bypass, the tendency to more resistance of erythrocytes to acid hemolysis in group 2, in comparison with group 1 was observed ($p = 0.05$).

The study of osmotic resistance of erythrocytes (ORE) showed that in the first and second groups before surgery, ORE parameters were not statistically significant ($p = 0.206$), and after CPB erythrocytes in group 2 were more resistant to hypoosmotic factor ($p = 0.01$). Decreased ORE is possible with glucose-6-phosphate dehydrogenase deficiency in erythrocytes and activation of lipid peroxidation [14]. The study in this direction continues.

CONCLUSIONS

In the research, it is confirmed that the use of the offered method (the treatment of the extracorporeal circuit by AdC) leads to the reduction of negative CPB impact on the condition of erythrocytes.

1. Electrophoresis showed that the treatment of the extracorporeal circuit with AdC leads to irreversible multicenter adsorption of the patient's autoalbumin on the working surface of the oxygenator (membranes and tubes) from a solution of low albumin concentration.

2. Tissue hypoxia and changes in the structure and function of erythrocytes are a leading pathogenetic factor in the development of critical conditions of patients who underwent open-heart surgery. Fewer echinocytes and spherocytes in blood samples after surgery in the group with the treatment of oxygenator surface with AdC indicates the more adequate oxygen supply to erythrocytes.

3. Hemolysis level in blood plasma after surgery was higher in the group without treatment of extracorporeal circuit with adaptation composition.

4. The intensity of hemolysis depends not only on mechanical damage of erythrocytes but also on a state of erythrocytes membrane after CPB. In the group where the extracorporeal circuit was treatment with AdC, membranes of erythrocytes were more resistant to the action of damaged factors.

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*Резюме***ВПЛИВ ОБРОБКИ ЕКСТРАКОРПОРАЛЬНОГО КОНТУРУ ОКСИГЕНАТОРА АДАПТУЮЧОЮ КОМПОЗИЦІЄЮ (AdC) НА МОРФОЛОГІЧНІ ЗМІНИ ЕРИТРОЦИТІВ****В.І. Черній¹, Л.О. Собанська¹, О.М. Лазаренко¹, Г.О. Лазаренко¹, Т.А. Алексєєва²**¹ Державна наукова установа «Науково-практичний центр профілактичної та клінічної медицини» Державного управління справами, Київ, Україна² Інститут хімії поверхні імені О.О. Чуйка Національної академії наук України, Київ, Україна

В роботі висвітлюється метод обробки екстракорпорального контуру оксигенатора «адаптуючою композицією» (adaptation composition – AdC) для зменшення негативного впливу штучного кровообігу на стан еритроцитів.

Мета дослідження: встановити вплив обробки контактної поверхні оксигенатора «адаптуючою композицією» (adaptation composition – AdC) на зміни стану еритроцитів при виконанні оперативних втручань на відкритому серці з використанням штучного кровообігу (ШК).

Матеріали і методи. В дослідження було включено 90 пацієнтів, які були розподілені на дві групи. Пацієнтам першої групи (45 хворих, 39/6 чол./жін.) оперативні втручання виконували без обробки контуру оксигенатора адаптуючою композицією (AdC). Оперативні втручання у пацієнтів другої групи (45 пацієнтів – 36/9 чол./жін.) проводились з обробкою AdC. Згідно протоколу дослідження, у пацієнтів набирали кров для загального аналізу крові та морфологічного дослідження еритроцитів на 4 етапах оперативного втручання: до початку операції, на 10 хв. ШК, на 60 хв. ШК та в кінці операції.

Результати та їх обговорення. Альбумін в складі AdC створює наночастинку на поверхні магістралей та мембрани оксигенатора. Між групами до ШК не було статистично значущої різниці у показниках. Рівень Ht 2 (група 2) на 60 хв. ШК ти після ШК був нижче, ніж Ht 1 (група 1) ($p=0,021$ і $p=0,035$ відповідно) через збільшення MCV 1 ($p=0,025$ і $p < 0,0001$ відповідно). На 10 хв. ШК підвищення MCHC в групах пов'язано зі зниженням MCV на 10 хв. ШК. На 60 хв. від початку ШК відбувається зміна в RDWa2 $76,05 \pm 5,46$ і RDWa1 $72,35 \pm 7,26$, $p < 0,0001$. Після ШК в групі 1 визначається більш високий вміст ретикулоцитів ($p < 0,0001$), ехіноцитів ($p < 0,0001$) і сфероцитів ($p < 0,0001$). В групі 1 після ШК відбувається зниження механічної стійкості еритроцитів ($p = 0,04$) та підвищена проникність мембрани еритроцитів для сечовини. Після ШК більша резистентність еритроцитів до кислотного гемолізу була в групі 2 ($p = 0,05$), також в групі 2 еритроцити були більш стійкими до гіпоосмотичному фактору ($p = 0,01$).

Висновки. Обробка оксигенатора адаптуючою композицією призводить до зменшення негативного впливу ШК на стан еритроцитів. Мембрани еритроцитів були більш резистентні до дії факторів що ушкоджують в групі з використанням AdC.

Ключові слова: штучний кровообіг, оксигенатор, морфологія еритроцитів, адаптуюча композиція.

Резюме**ВЛИЯНИЕ ОБРАБОТКИ ЭКСТРАКОРПОРАЛЬНОГО КОНТУРА ОКСИГЕНАТОРА АДАПТИРУЮЩЕЙ КОМПОЗИЦИЕЙ (AdC) НА МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ЭРИТРОЦИТОВ****В.И. Черний¹, Л.О. Собанская¹, О.Н. Лазаренко¹, Г.О. Лазаренко¹, Т.А. Алексеева²**¹ Государственное научное учреждение «Научно-практический центр профилактической и клинической медицины» Государственного управления делами, Киев, Украина² Институт химии поверхности имени А.А. Чуйко Национальной академии наук Украины, Киев, Украина

В работе освещается метод обработки экстракорпорального контура оксигенатора «адаптирующей композицией» (adaptation composition – AdC) для снижения негативного влияния искусственного кровообращения на состояние эритроцитов.

Цель исследования: установить влияние обработки контактной поверхности оксигенатора «адаптирующей композицией» (AdC) на изменения состояния эритроцитов при выполнении оперативных вмешательств на открытом сердце с применением искусственного кровообращения (ИК).

Материалы и методы. В исследование было включено 90 пациентов, которые были распределены на две группы. Пациентам первой группы (45 больных, 39/6 муж. /жен.) оперативные вмешательства выполняли без обработки контура оксигенатора адаптирующей композицией (AdC). Согласно протоколу исследования, у пациентов набирали кровь для общего анализа крови и морфологического исследования эритроцитов на 4 этапах оперативного вмешательства: до начала операции, на 10 мин. ИК, на 60 мин. ИК и в конце операции.

Результаты и их обсуждение. Альбумин в составе AdC создает нанослой на поверхности мембраны оксигенатора. Между группами до ИК не было статистически значимой разницы между показателями. Уровень Ht 2 (группа 2) на 60 мин. ИК и после ИК был ниже, чем Ht 1 (группа 1) ($p=0,021$ и $p=0,035$ соответственно) за счет увеличения MCV1 ($p=0,025$ и $p<0,0001$ соответственно). На 10 мин. ИК повышение MCHC в группах связано со снижением MCV на 10 мин ИК. На 60 мин. от начала ИК происходит изменение RDWa2 $76,05 \pm 5,46$ и RDWa1 $72,35 \pm 7,26$, $p<0,0001$. После ИК в группе 1 отмечается более высокое содержание ретикулоцитов ($p<0,0001$), эхиноцитов ($p<0,0001$) и сфероцитов ($p<0,0001$). В группе 1 после ИК происходит снижение механической стойкости эритроцитов ($p=0,04$), и повышение проницаемости мембраны эритроцитов для мочевины. После ИК большая резистентность эритроцитов к кислотному гемолизу была в группе 2 ($p=0,05$), также в группе 2 эритроциты были более устойчивыми к гипосмотическому фактору ($p=0,01$).

Выводы. Обработка оксигенатора адаптирующей композицией приводит к уменьшению негативного влияния ИК на состояние эритроцитов. Мембраны эритроцитов были более резистентными к действию повреждающих факторов в группе с AdC.

Ключевые слова: искусственное кровообращение, оксигенатор, морфология эритроцитов, адаптирующая композиция.

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