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INFLUENCE OF CARDIOPULMONARY BYPASS ON THE ERYTHROCYTE MEMBRANES AND THE METHOD OF ITS PROTECTION

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Abstract. Influence of cardiopulmonary bypass on the erythrocyte membranes and the method of its protection. Cherniy V.I., Sobanska L.O., Topolov P.O., Cherniy T.V. *The damage to erythrocytes during cardiopulmonary bypass (CPB) remains a recent problem. The aim of this research was to study the effect of fructose-1,6-diphosphate on the state of the erythrocyte membrane during CPB and the level of phosphorus in blood as a marker of the energy potential in the cell. Patients were divided into two groups. The control group 1 (Gr 1) consisted of 75 individuals. The group 2 (Gr 2) included patients to whom fructose-1,6-diphosphate (FDP) was administrated according to the developed scheme as follows 10 g of the drug was diluted in 50 ml of a solvent, 5 g of the drug was injected intravenously with the use of perfusor immediately before initiation of CPB at a rate of 10 ml/min and 5 g at the 30th minute of CPB (before the stage of warming) the same way. When comparing two groups the best results in hemolysis (p<0.01), mechanical (p<0.01). osmotic resistance of erythrocytes (p<0.01), the time of acid hemolysis (p<0.01) and the permeability of the erythrocyte membrane in postperfusion period were in Gr 2. Before cardiac surgery hypophosphatemia was detected in 18% out of 150 and in 32% out of 150 patients – a lower limit of normal phosphorus content in the blood. After CPB in Gr 1 phosphorus content in blood was 0.85±0.32 mmol/l and hypophosphatemia was in 53% out of 75 patients. This indicates a pronounced energy deficit in this group. In Gr 2 phosphorus level was 1.7±0.31 mmol/l and there was no hypophosphatemia. As a result, FDP as an endogenous high-energy intermediate metabolite of the glycolytic pathway leads to resistance to hemolysis, protects the erythrocyte membrane from damage and increases the energy potential of the cell during CPB.*

Реферат. Вплив штучного кровообігу на мембрани еритроцитів і спосіб їх захисту. Черній В.І., Собанська Л.О., Тополов П.О., Черній Т.В. *Пошкодження еритроцитів при штучному кровообігу (ШК) залишається актуальною проблемою. Метою дослідження було вивчення впливу фруктозо-1,6-дифосфату на стан мембрани еритроцитів під час ШК і рівень фосфору в крові як маркера енергетичного потенціалу клітини. Пацієнти були розподілені на дві групи. Контрольну групу 1 (Gr 1) склали 75 осіб. До групи 2 (Gr 2) увійшли пацієнти, яким вводили фруктозо-1,6-дифосфат (ФДФ) за розробленою нами схемою таким чином: 10 г препарату розводили в 50 мл розчинника, 5 г препарату вводили внутрішньовенно з використанням перфузора безпосередньо перед початком ШК зі швидкістю 10 мл/хв і 5 г на 30-й хвилині ШК (перед етапом зігрівання) аналогічним чином. При порівнянні двох груп кращі результати гемолізу (p<0,01), механічної (p<0,01), осмотичної резистентності еритроцитів (p<0,01), часу кислотного гемолізу (p<0,01) і проникності мембран еритроцитів у постперфузійному періоді були в Gr 2. До операції на серці гіпофосфатемія була виявлена у 18% зі 150 пацієнтів і в 32% зі 150 пацієнтів виявлено нижню межу нормального вмісту фосфору в крові. Після ШК у Gr 1 вміст фосфору в крові становив 0,85±0,32 ммоль/л, а гіпофосфатемія була в 53% з 75 пацієнтів. Це свідчить про виражений енергетичний дефіцит у цій групі. У Gr 2 рівень фосфору був*

1,7±0,31 ммоль/л, а гіпофосфатемії не було. У результаті ФДФ як ендогенний високоенергетичний проміжний метаболіт гліколітичного шляху збільшує резистентність до гемолізу, захищає мембрану еритроцита від ушкодження і збільшує енергетичний потенціал клітини під час ШК.

The normal function of erythrocyte membrane and energy potential of the cell during cardiopulmonary bypass is of the essence. Changing the state of cell membranes can serve as an early signal of the development of pathological processes. The state of erythrocytes and their deformability largely depends on the intracellular content of adenosine triphosphate (ATP): decreasing ATP level leads to reduced deformability, and increasing ATP level leads to increased deformability [12, 15]. Hypophosphatemia is one of the disturbance mechanisms of energy supply of intracellular homeostasis processes in erythrocytes and it is often found in cardiac surgery patients, patients of intensive care units, especially in patients on mechanical ventilation [11, 13]. There is evidence that insulin resistance and associated hyperglycemia after cardiac surgery is the result of hypophosphatemia [10]. Fructose-1,6-diphosphate (FDP) is an endogenous high-energy intermediate metabolite of the glycolytic pathway that increases ATP production and has an organoprotective effect in various pathological conditions associated with oxygen deficiency. Increasing the concentration of erythrocyte ATP leads to improved blood rheology and resistance to hemolysis due to better deformability of erythrocytes [8, 12]. An effective way to optimize energy metabolism under hypoxic conditions is exogenous delivery of FDP to the patient.

The measurement of plasma free hemoglobin (plfHb) is a well-known method but it does not define sublethal trauma of RBC. The concept of sublethal RBC damage was introduced by Dr. Galletti [14]. This is especially important to consider in the process of cardiopulmonary bypass (CPB). A reasonable way to assess the structural and functional state of erythrocytes is to determine the resistance of blood cells to mechanical, osmotic, and acidic factors [4]. The purpose of the research is to study and reduce the damaging effect of CPB on an erythrocyte membrane and improve energy potential of erythrocytes during CPB.

MATERIALS AND METHODS OF RESEARCH

The patients were divided into two groups. The first group (Gr 1, n=75) was the control group, the second group (Gr 2, n=75) included individuals who were administered the drug with the active substance FDP before and during CPB. The distribution of patients into groups is presented in table 1.

Table 1

The distribution of patients into groups

Characteristics	Gr1 (n=75) *	Gr2 (n=75) *
Gender: male	61	62
female	14	13
Age (M±m), years	63.05±8,89	63.39±9.34
Weight (M±m), kg	87.67±16.41	85.7±11.48
NYHA** functional class: Class II	6 (8.0%)	5 (6.7%)
Class III	57 (76.0%)	56 (74.7%)
Class IV	12 (16.0%)	14 (18.6%)

Notes: * – the difference in parameters in groups by test $\chi 2$ statistically is not significant ($p>0.05$); NYHA ** – New York Heart Association Classification.

113 (75.3%) patients underwent coronary artery bypass grafting (CABG), 10 (6.7%) patients underwent CABG + left ventricular aneurysm resection (LVAR), 13 (8.6%) patients underwent aortic valve replacement (AVR), 3 (2%) patients underwent AVR+ CABG, 5 (3.4%) patients underwent mitral valve replacement (MVR), 6 (4%) – MVR+CABG. Management during on-pump CABG surgery includes aortic cross-clamping followed by fibrillation and aortic cross-clamping followed by crystalloid cardioplegia during aortic and mitral valve replacement. The cardiopulmonary bypass time (CPB-time) in Gr 1 was 98.4±19.8 min., in Gr 2 – 93.85±19.54 min. The perfusion system used a membrane oxygenator, roller pump, nonpulsatile flow, and the primed circuit 1.3-1.6 l to achieve

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moderate hemodilution (Ht – 25±2 г/л). Hyperosmolar prime volume with an osmolarity of 510.9 mosmol/l was used [6]. The mean flow index 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 (32-33ºC). This study complied with the ethics committee approval, written informed consent was obtained from patients. Exclusion criteria included: hereditary fructose intolerance, creatinine clearance below 50 ml/min, hypernatremia, hyperphosphatemia.

In Gr 2 the dosage regimens of FDP were as follows: 10 g of the drug was diluted in 50 ml of a solvent, 5 g of the drug was injected intravenously with the use of perfusor immediately before initiation of CPB at a rate of 10 ml/min and 5 g at the 30th minute of CPB (before the stage of warming) in the same way [7]. According to the study protocol, patient's blood was sampled for erythrocyte resistance and phosphorus level research before surgery and after CPB. Several parameters were studied. Plasma free hemoglobin (plfHb) concentration was measured using the hemoglobin cyanide method [5]. Erythrocytes osmotic resistance was carried out by the method of determining the time up to 50% hemolysis of a blood sample in a buffer hypotonic glycerol-saline mixture in one tube [9]. The method of Y.V. Ganitkevich, L.I. Chernenko was used for mechanical resistance of erythrocytes [4]. The result was expressed as % of hemolyzed cells after mechanical exposure. Erythrocyte membrane permeability (EMP) was determined using the method of urea hemolysis [2]. The concentration of urea in a series of buffered hypotonic solutions was increased and the degree of hemolysis was studied. Acid hemolysis was determined by I.A. Terskov and I.I. Gitelzon [3].

«MedStart» software program was used for the statistical analyses (licence certificate v. 4. MS 00070-06.07.2009, Y.Y. Liakh, V.G. Gurianov). We checked data for normality before further analysis and used mean values, standard error, Student's t-test. The $\chi 2$ (Pearson) criterion was used to assess the statistical significance of the differences between two or more relative data. Group differences were considered statistically significant at p-value <0.05 [1].

RESULTS AND DISCUSSION

Before surgery hypophosphatemia was detected in two groups: 18% of patients (n=27) have hypophosphatemia and 32% of patients have a clear tendency to it (49 patients have a lower limit of normal phosphorus content in the blood), indicating an initial energy deficiency in this category of patients. The content of phosphorus before CPB in Gr 1 (1.13±0.22) and Gr 2 (1.16±0.22) was comparable (p=0.454). In Gr 2 phosphorus levels (1.7±0.31) were statistically significantly higher after surgery (p<0.01) and there was no hypophosphatemia. In Gr 1 the phosphorus level in the blood (0.85±0.32) after surgery decreased significantly compared with the baseline (p<0.01). After CPB in Gr 1 hypophosphatemia was in 53% out of 75 patients. Analysis results after CPB showed significantly decreased phosphorus level in Gr 1 compared with Gr 2 (p<0.01).

Table 2

Phosphorus value, hemolysis, erythrocyte resistance in Gr1 and Gr 2 before and after CPB

Parameters		Mean±SD		p*
		Gr 1 (n=75)	Gr 2 (n=75)	
Phosphorus, mmol/l	Before CPB	1.13±0.22	1.16±0.22	0.45
	After CPB	0.85±0.32	1.7±0.31	p<0.01
Hemolysis, g/l	Before CPB	0.15±0.08	0.16±0.09	0.632
	After CPB	0.57±0.23	0.44±0.15	p<0.01
Mechanical resistance of erythrocytes, %	Before CPB	58.62±19.8	53.16±16.96	0.29
	After CPB	79.83±15.68	68.88±15.56	p<0.01
Time of acid hemolysis 50% of erythrocytes, sec.	Before CPB	228.1±36.49	232.6±41.96	0.90
	After CPB	132.9±33.04	151.3±31.33	p<0.01
Osmotic resistance of erythrocytes, sec.	Before CPB	456.9±239.7	501.1±240.6	0.62
	After CPB	247.3±129.4	362.4±179.9	p<0.01

Notes: * – p<0.05 reliability of indicators between Gr 1 and Gr 2; CPB – cardiopulmonary bypass.

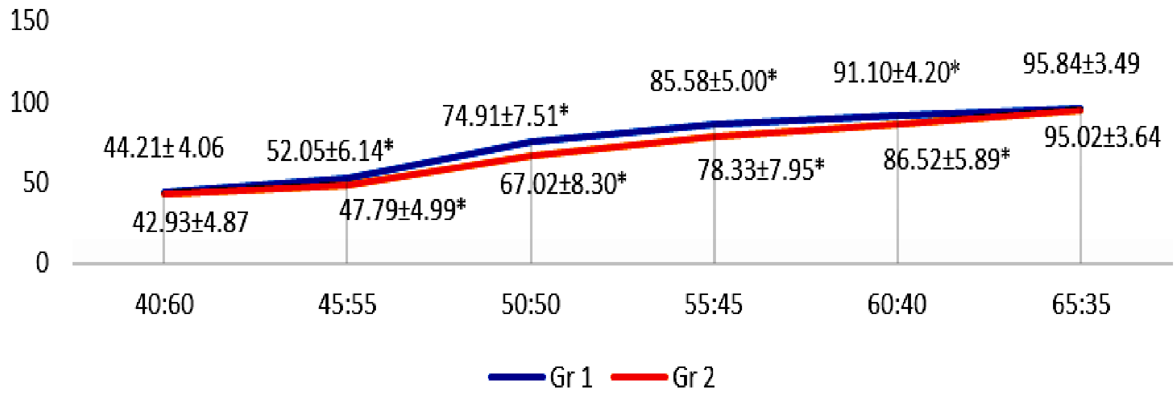
Hemolysis during extracorporeal circulation is the result of the destruction of the RBC membrane with the breakdown and release of plasma free hemoglobin. There are no significant differences in the level of hemolysis between groups before CPB (p=0.632). After CPB hemolysis was higher in Gr 1 (p<0.01).

After CPB there was a greater decrease in the mechanical resistance of erythrocytes in Gr 1 compared with Gr 2 (p<0.01).

Acid resistance of erythrocytes allows judging about condition of a phospholipid bilayer and proteins of membranes of erythrocytes [4]. After CPB, there was a tendency for greater resistance of erythrocytes to acid hemolysis in Gr 2 (p<0.01).

The study of osmotic resistance of erythrocytes (ORE) showed that after CPB erythrocytes in Gr 2 were more resistant to hypoosmotic factor (p=0.01).

Assessment of the erythrocyte membrane permeability (EMP) for the urea solution revealed that there was no statistical difference in urea hemolysis between Gr1 and Gr2 before CPB. After CPB (Fig.) where the hypotonic solutions of urea and sodium chloride were diluted in a ratio of 45:55, there was a tendency to a higher level of erythrocyte hemolysis in Gr 1 (p=0.05). When the solution was diluted in a ratio of 50:50, 55:45 (p<0.01), 60:40 (p=0.01) this tendency persisted. At a dilution of 65:35, almost all erythrocytes were lysed in two groups and there was no statistical difference in data (p=1.0).



Note: * – the difference in parameters is statistically significant (p<0.05)

Parameters of erythrocyte membrane permeability (EMP %) for urea solution after CPB

Studies have shown that a lower level of hemolysis, better resistance to mechanical hemolysis, ORE, EMP, and acid hemolysis in Gr 2 are due to the protection of FDP cells from physical and chemical damaging factors. A decrease in ORE is possible with a deficiency of ATP in erythrocytes and activation of lipid peroxidation [12, 13].

CONCLUSIONS

1. In cardiac surgery patients it was found that before CPB hypophosphatemia or the tendency to the lower limit of normal phosphorus content in the blood were determined in 50% of cases. Hypophosphatemia is one of the mechanisms of impairment of energy supply of processes in erythrocytes.
2. The administration of FDP according to the developed scheme led to the correction of hypophosphatemia in Gr 2 and there was no hypophosphatemia after CPB. In Gr1 hypophosphatemia was in 53% out of 75 patients after perfusion.
3. Hemolysis that developed after extracorporeal circulation as a result of damage to the erythrocyte membrane was higher in Gr 1 compared to Gr 2 (p<0.01).
4. After CPB lower mechanical and osmotic resistance of erythrocytes, the time of acid hemolysis of 50% of erythrocytes, and the increased permeability of the erythrocyte membrane in Gr 1 indicate significant damage to the erythrocyte membrane and a decrease in its resistance to cardiopulmonary bypass compared to Gr 2.
5. It was found that the administration of FDP according to the developed scheme (5 g of the drug was injected intravenously through a syringe dispenser immediately before the start of perfusion at a rate of 10 ml/min and 5 g before the stage of warming) increases the resistance of the erythrocyte membrane to the action of traumatic factors. In Gr 2 there was a lower level of hemolysis and better erythrocyte resistance in comparison with control Gr 1.

Conflict of interests. The authors declare no conflict of interest.

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