DOI: http://doi.org/10.17816/2313-8726-2024-11-1-69-76

Influence of the program for the accelerated recovery of postpartum women on the microviscosity of the erythrocyte membranes after cesarean section

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ABSTRACT

Background. This study aimed to analyze the microviscosity of membranes and the surface charge of erythrocytes and blood plasma proteins using fluorescent zones in pregnant women before and after cesarean section (CS) with traditional management of the perioperative period and use of an accelerated recovery program.

Materials and methods. The study included pregnant women without somatic or obstetric complications. They were divided into groups that received traditional management during the perioperative period (fasting for >8 h before surgery and administration of an antibiotic after clamping the umbilical cord) and used an accelerated recovery program. The relative microviscosity of the erythrocyte membranes was assessed using the lateral diffusion method with a hydrophobic pyrene probe (C16H10). The surface charge of erythrocytes and blood plasma proteins was determined using the fluorescent probe 8-anilino-1-naphthalene sulfonic acid.

Results. In postpartum women with an accelerated recovery program, the immersion of proteins in the lipid bilayer of the erythrocyte membrane decreased. The coefficient of pyrene eximerization in areas of protein–lipid contacts in the same group was higher and further increased both after anesthesia and after surgery. In the traditional management group, an increase in the eximerization coefficient was registered only after CS. In the areas of lipid–lipid contacts, the coefficient of pyrene eximerization in the accelerated recovery program group was significantly higher than that in the control group. Changes in the eximerization coefficient after anesthesia and surgery were insignificant. Moreover, the polarity of the microenvironment of both anular lipids and total lipids did not change.

Conclusions. An increase in the pyrene eximerization coefficient in patients on an accelerated recovery program indicates a decrease in the microviscosity of erythrocyte membranes and an increase in their fluidity, which may be due to structural rearrangements of cell membranes and leads to an increase in their functional activity. The components of the accelerated recovery program affect the microviscosity of erythrocyte membranes, particularly in the areas of protein–lipid contacts, which may positively affect microcirculation in the perioperative period, which plays a significant role in the genesis of postoperative complications.

Keywords: fluorescent probe ANS (8-anilino-1-naphthalene sulfonic acid); blood plasma proteins; cesarean section; membrane microviscosity; accelerated recovery program; pyrene; kinetic parameters; fluorescent probes; fast track; accelerated recovery program; perioperative period.

To cite this article:
Medzhidova DR, Shifman EM, Cherkesova AU, Cherkesova DU. Influence of the program for the accelerated recovery of postpartum women on the microviscosity of the erythrocyte membranes after cesarean section. *V.F. Snegirev Archives of Obstetrics and Gynecology.* 2024;11(1):69–76. doi: 10.17816/2313-8726-2024-11-1-69-76

Received: 23.10.2023 **Accepted:** 31.01.2024 **Published:** 27.03.2024

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DOI: http://doi.org/10.17816/2313-8726-2024-11-1-69-76

Влияние программы ускоренного выздоровления родильниц после операции кесарева сечения на состояние микровязкости эритроцитарных мембран

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АННОТАЦИЯ

Введение. Цель исследования — изучение микровязкости мембран и поверхностного заряда эритроцитов и белков плазмы крови с использованием флуоресцентных зон у беременных до и после кесарева сечения (КС) при традиционном ведении периоперационного периода и с использованием программы ускоренного восстановления.

Материалы и методы. В исследование включены беременные без соматических и акушерских осложнений, из них сформированы группы с традиционным ведением периоперационного периода (голодание более 8 часов до операции, введение антибиотика после пережатия пуповины) и с использованием программы ускоренного восстановления. Оценку относительной микровязкости мембран эритроцитов осуществляли методом латеральной диффузии гидрофобного зонда пирена (C16H10). Поверхностный заряд эритроцитов и белков плазмы крови определяли с помощью флуоресцентного зонда АНС (8-анилино-1-нафталинсульфоновая кислота).

Результаты. У родильниц с программой ускоренного восстановления выявлено снижение погружённости белков в липидный бислой мембраны эритроцитов. Коэффициент эксимеризации пирена в зонах белок–липидных контактов в этой же группе пациенток оказался выше и далее возрастал как после анестезии, так и после операции. У пациенток с традиционной подготовкой отмечается повышение коэффициента эксимеризации только после КС. В зонах липид–липидных контактов коэффициент эксимеризации пирена у женщин с применением программы ускоренного восстановления достоверно выше, чем в сравниваемой группе контроля. Изменения коэффициента эксимеризации после анестезии и операции незначительны. При этом не обнаружены изменения полярности микроокружения как анулярных липидов, так и общих липидов.

Заключение. Повышение коэффициента эксимеризации пирена у пациенток с программой ускоренного восстановления указывает на снижение микровязкости мембран эритроцитов, увеличение их текучести, что может быть обусловлено структурными перестройками клеточных мембран, приводящими к усилению их функциональной активности. Компоненты программы ускоренного восстановления оказывают влияние на микровязкость мембран эритроцитов особенно в зонах белок–липидных контактов, что возможно положительно сказывается на микроциркуляции в периоперационном периоде, то есть факторе, играющем значительную роль в генезе послеоперационных осложнений.

Ключевые слова: флуоресцентный зонд АНС (8-анилино-1-нафталинсульфоновая кислота); белки плазмы крови; кесарево сечение; микровязкость мембран; программа ускоренного выздоровления; пирен; кинетические параметры; флуоресцентные зонды; фаст трек; программа ускоренного восстановления; периоперационный период.

Как цитировать:
Меджидова Д.Р., Шифман Е.М., Черкесова А.У., Черкесова Д.У. Влияние программы ускоренного выздоровления родильниц после операции кесарева сечения на состояние микровязкости эритроцитарных мембран // Архив акушерства и гинекологии им. В.Ф. Снегирёва. 2024. Т. 11, № 1. С. 69–76. doi: 10.17816/2313-8726-2024-11-1-69-76

Рукопись получена: 23.10.2023 **Рукопись одобрена:** 31.01.2024 **Опубликована:** 27.03.2024

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INTRODUCTION

Choosing the best clinical approaches to minimize the risk associated with abdominal delivery and surgical complications remains a major concern [1-2]. Surgical complications following cesarean section (CS) may be attributed to impaired blood circulation, oxygen deficiency in tissues and organs, hormonal and metabolic dysregulation, free radical oxidation (FRO), and oxidative stress.

In a previous study, we have reported that cytotoxic end-products of lipid peroxidation (LP) are able to accumulate in erythrocyte membranes post-surgery [3]. Overproduction of free radicals results in structural and functional integrity defects of cell membranes, impairing their dynamic properties and lipid bilayer fluidity. Furthermore, increased membrane microviscosity, as well as changes in permeability and osmotic resistance may be observed [4-5].

Erythrocytes are regarded as an integral indicator of cell membrane performance, and the structural and functional properties of erythrocyte membranes have been extensively studied under both normal and abnormal conditions [6-7].

In recent years, fluorescent probes (FPs) have been employed to investigate a range of physical properties, including those of membrane lipids, protein conformations, and other cell components. They have also been used to examine the molecular mechanisms underlying pathological processes, the effects of drugs, and the efficacy of therapies [4, 5, 8-10].

There is a variety of task-specific classes of FPs [4, 5, 7].

A comprehensive review of the scientific literature reveals a paucity of research on the use of FPs in obstetrician practice.

The objective of this study was to investigate erythrocyte membrane microviscosity using a pyrene-based FB and a surface charge of erythrocytes and plasma proteins. An ANS-based (8-aniline-1-naphthalenesulfonic acid) FP was used in pregnant women before and after CS with the conventional perioperative management or enhanced recovery program (ERP) technology.

MATERIALS AND METHODS

The study included pregnant women with no history of somatic and obstetric complications who had elective abdominal delivery. All patients signed an informed consent to participate in the study. The study was approved by the Local Ethics Committee of the Dagestan State Medical University (Protocol No. 23 of April 17, 2018).

The patients were divided into two groups: Group 1 (control group) for the conventional perioperative management (fasting for >8 hours before surgery and antibiotic administration following cord clamping) (n=41); and Group 2 (treatment group) for the ERP technology (high-carbohydrate enteral formula 2 hours prior to surgery and antibiotic administration 1 hour prior to surgery) (n=41). Blood samples were taken in both groups before and after anesthesia, as well as after surgery. Blood was collected from the ulnar vein into heparin tubes, refrigerated, and centrifuged at 1,500 rpm for 5 minutes in an Eppendorf 5702R centrifuge to separate plasma from erythrocytes, as previously described [3]. Erythrocyte membranes were isolated from precipitated erythrocytes. Achromacytes were obtained according to Klenova et al. [6].

The relative microviscosity of blood cell membranes was assessed by lateral diffusion of a pyrene-based hydrophobic probe (C16H10) [8-9]. The microviscosity of erythrocyte membranes was measured using a Hitachi F7000 Fluorescence Spectrophotometer (Japan) according to Panin et al. [11].

The microviscosity of the lipid layer in erythrocyte membranes was measured at excitation wavelength 337 nm (with the spectral slit-width of 1.5/2.5). The microviscosity of protein–lipid interfaces was measured at a maximum of 280 nm (with the spectral slit-width of 1.5/5). Maximum fluorescence wavelengths were 395 nm for pyrene monomers and 470 nm for excimers.

Structural rearrangements of membrane proteins were assessed by the efficiency of excitation energy transfer between intrinsic tryptophan residues and pyrene. The quenching of membrane protein fluorescence by pyrene was measured at $\lambda_{excitation}$=280 nm and $\lambda_{emission}$=333 nm. The efficiency of excitation energy transfer was determined by the formula $(F_0–F/F_0)\times100$, where F_0 is the fluorescence intensity of a pyrene-free suspension of erythrocyte membranes; F is the fluorescence intensity of a suspension of erythrocyte membranes incubated with pyrene (7.76 μm) [8-9].

The polarity of the lipid bilayer and protein–lipid interfaces in erythrocyte membranes was identified by the fluorescence intensity ratio of two monomers (F_{374}/F_{394}) in the fine structure of pyrene at $\lambda_{excitation}$ 337 nm and 280 nm, respectively.

The surface charge of erythrocytes and plasma proteins was determined using an ANS-based fluorescent probe [8]. The Hitachi F7000 Fluorescence Spectrophotometer was used to measure fluorescence at $\lambda_{excitation}$=360 nm and $\lambda_{emission}$=480 nm (native fluorescence). The concentration-dependent fluorescence of ANS incubated with erythrocytes and blood plasma was analyzed within the range of probe concentrations 0.005–0.05 μM at 25 °C. The maximum fluorescence intensity was determined by ANS fluorescence spectra. Then, the maximum fluorescence intensity (F) was plotted against the probe concentration in the sample, and kinetic characteristics were calculated using nonlinear multidimensional regression analysis and non-linear Michaelis—Menton equation.

Statistical analysis was performed using the STATISTICA software package. The tabulated values represent the mean of 20 independent experiments. The significance of differences was assessed using the Student's t-test.

RESULTS

Pregnant women's erythrocyte membranes have been shown to have higher levels of LP end-products following

anesthesia and CS. This observation appears to align with developing oxidative stress [3]. It was interesting to investigate how elevated levels of cytotoxic free radicals impact the structural and functional properties of erythrocyte membranes. It is well established that the cytoplasmic membrane plays a pivotal role in cell functions. It is responsive to a multitude of external and internal factors, exhibiting alterations in its dynamic properties, lipid bilayer fluidity, and microviscosity [7, 9]. Erythrocyte membranes serve as an integral indicator of cell membrane performance under abnormal conditions, as well as a reliable indicator of oxidative stress and total antioxidant status.

The findings obtained with FPs are presented in Tables 1 and 2.

The analysis of membrane protein immersion into the lipid bilayer using pyrene-based FPs showed no significant differences between the compared groups of pregnant women. In the conventional approach to CS management, there was only a slight reduction in the protein immersion into the lipid bilayer following anesthesia and surgery. In contrast, the ERP group demonstrated a 11.4% and 11.7% decrease in protein immersion, respectively. This finding suggests the potential for structural and functional rearrangements of membranes.

To elucidate the causal relationship between the observed changes, the membrane microviscosity at the protein-lipid and lipid-lipid interfaces was analyzed.

The results demonstrated that in the ERP group, the pyrene excimerization coefficient (Kex) at the protein-lipid interfaces of erythrocyte membranes was 15.7% higher compared to that for the conventional perioperative management. This indicated a decrease in the microviscosity of erythrocyte membranes and an increase in fluidity at the protein-lipid interfaces. It is evident that the difference in Kex values of pyrene between the compared groups is attributable to the ERP components. After anesthesia and surgery, there was a tendency for an increase in Kex in both groups. The conventional perioperative management was associated with a 23% postsurgical increase in Kex for pyrene, while the ERP group exhibited an increase in Kex after anesthesia and surgery by 11.4% and 25%, respectively.

Apart from the ERP components, the CS procedure obviously affects the microviscosity of the protein-lipid interfaces of the membranes, contributing to higher fluidity and lower protein immersion into the lipid bilayer. This phenomenon may be attributed to the modification of cell-surface membrane proteins resulting from FRO activation, as has been previously demonstrated [3].

The analysis of the lipid bilayer microviscosity has shown that in the ERP group, the Kex value for pyrene at the lipid-lipid interfaces of erythrocyte membranes was significantly higher than that in the control group. However, Kex variations induced by anesthesia and surgical procedure were insignificant in both groups. It can be concluded that the ERP components also contribute to a reduction in the lipid bilayer microviscosity and an increase in fluidity. However, the effects of anesthesia and CS on the lipidlipid interfaces of erythrocyte membranes are less pronounced than their impact on the protein-lipid interfaces.

It is known that a change in the polarity of the lipid bilayer and anular lipids may result in binding defects, membrane "gaps," and dysfunction [5].

We found no significant changes in the polarity of the microenvironment of both protein-adjacent anular lipids characterized by better organization, lower mobility, and higher levels of saturated fatty acids, and total lipids (see Table 1).

ANS-based probe fluorescence is a useful method for assessing the modification of the surface charge of erythrocytes and plasma proteins [8]. To analyze the erythrocyte-ANS binding kinetics, we investigated the ANS fluorescence intensity against concentration after probe incubation with erythrocyte membranes and blood plasma (Table 2).

The data presented in Table 2 suggest that there are specific characteristics and diverse trends associated with the number of ANS binding sites for surface proteins of

Table 1. The structural state of the membranes of erythrocytes of the blood of maternity hospitals

Blood samples	Protein immersion into lipid bilayer (Fo–F)/Fo, %	Microviscosity of lipid-protein interfaces Fe/Fm (λ=280)	Microviscosity of lipid layer Fe/Fm (λ=337)	Polarity of anular lipid microenvironment F370/F390 (λ=280)	Polarity of total lipid microenvironment F370/F390 (λ=337)
Group 1 (control)	47,5±1,83	0,38±0,034	0,44±0,040	1,05±0,090	0,90±0,07
Post-anesthesia	47,81±2,33	0,40±0,027	0,46±0,038	1,04±0,086	0,91±0,074
Post-surgery	45,24±1,83	0,47±0,028*	0,45±0,041	1,03±0,084	0,93±0,08
Group 2 (antibiotic + glucose)	48,65±1,85	0,44±0,03	0,55±0,036*	1,02±0,090	0,91±0,076
Post-anesthesia	43,11±1,44*	0,49±0,035*	0,57±0,048*	1,03±0,093	0,90±0,071
Post-surgery	42,93±1,56*	0,55±0,055*	0,55±0,037*	1,04±0,10	0,89±0,07

Note. Fe/m is the ratio of excimer fluorescence intensity to monomer fluorescence; *the differences between the indicators of the main and control groups of patients were significant (p <0.05).

Table 2. Kinetic parameters of binding of ANS (8-anilino-1-naphthalenesulfonic acid) to plasma proteins and erythrocytes of the blood of maternity women

Blood samples	Blood plasma		Erythrocytes	
	N, binding sites/mg of protein	Kd, mmol	N, binding sites/mg of protein	Kd, mmol
Group 1 (control)	10466±340	0,0132±0,0011	867±36	0,0705±0,005
Post-anesthesia	9807±310	0,010±0,001*	980±42*	0,0704±0,005
Post-surgery	9592±260*	0,0096±0,001*	992±0,47*	0,0547±0,005*
Group 2 (antibiotic + glucose)	8682±385 p_{1-5}<0,05	0,081±0,0012 p_{1-5}<0,003	995±47 p_{1-5}<0,05	0,0732±0,005
Post-anesthesia	8417±390*	0,085±0,0012*	1168±76*	0,0577±0,005*
Post-surgery	8176±455*	0,089±0,001*	1188±80*	0,0545±0,005*

Note. Kd, mM is the dissociation constant; *the differences between the indicators of the main and control groups of patients were significant (p <0.05).

erythrocytes and blood plasma. In the ERP group, there was a 14.7% increase and a 17.1% decrease in fluorescence after ANS incubation with erythrocytes and blood plasma, respectively, compared to the control group. The contribution of free ANS to the fluorescence intensity is known to be negligible, and the increase in fluorescence is attributed to emission associated with the positive surface charge of erythrocytes [8]. The negative charge inversely correlates with the fluorescence intensity. Based on these findings, there is an increase in the positive charge of erythrocyte surface proteins and a decrease in that for blood plasma, which can be attributed to the potential modification of protein molecules as a result of the ERP components.

Following anesthesia and CS, both groups showed similar changes. These included an increase in the number of ANS binding sites for surface proteins of erythrocytes and a decrease in binding to blood plasma proteins. However, the most significant changes after anesthesia and CS were found in erythrocytes for the ERP group (an increase in fluorescence by 17.4% and 19.4% compared to 13% and 14.4% for the conventional perioperative management). There was a decrease in the number of ANS binding sites in plasma in both groups. This tendency was more significant post-surgery in patients from the conventional management group.

The analysis of the relationship between ANS fluorescence in erythrocyte samples and concentration showed a decrease in the dissociation constant (Kd, mmol) in both groups post-surgery. This suggests an increase in the apparent number of probe binding sites. The dissociation constant decreased by 25.5% for the ERP group and by 22% for the conventional perioperative management group. The blood plasma dissociation constant exhibited a variety of trends in the compared groups. The conventional management was associated with a decrease in Kd after anesthesia (by 6.3%) and CS (by 8.4%), whereas in the ERP group, the values tended to increase post-surgery (by 9.9%). This suggests a decrease in the apparent number of probe binding sites as a result of the ERP components.

Our findings indicate that the ERP components had an effect on the microviscosity of erythrocyte membranes, increasing their fluidity at the protein-lipid and lipid-lipid interfaces. After anesthesia and surgery, an increase in microviscosity was largely observed at the protein-lipid interfaces of erythrocyte membranes in the compared groups. Obviously, despite the LP activation previously identified in the study groups [3], free radicals have no significant impact on the structural and functional integrity of the lipid bilayer. Rather, they result in the modification of anular lipids of erythrocyte membranes, with only insignificant variations in the polarity of the microenvironment of anular and total lipids. The observed changes in erythrocyte membrane dynamics, which result in a reduction in the viscosity of anular lipids, may be regarded as an adaptive response to the ERP components and CS. This response is beneficial for microcirculation during the perioperative period, which is a major contributor to postoperative complications.

A significant increase in the number of ANS binding sites for erythrocyte surface proteins in the ERP group, followed by their increase after anesthesia and CS, as well as a decrease in the number of binding sites for plasma proteins, suggests that the ERP components may be responsible for the structural modifications of membrane proteins and plasma proteins.

CONCLUSIONS

1. The pyrene-based FP has demonstrated similarities in the protein immersion into the lipid bilayer of erythrocyte membranes between the control group with conventional preparation for cesarean section and the group using the ERP technology. Following anesthesia and CS, the protein immersion into the lipid bilayer of erythrocyte membranes

in the control group remained almost unchanged, whereas it decreased in the ERP group.

2. The ERP components contributed to a decrease in the microviscosity of erythrocyte membranes at the protein-lipid and lipid-lipid interfaces in the preoperative period. In both groups, CS resulted in a decrease in the microviscosity of erythrocyte membranes only at the protein-lipid interface.

3. The ERP components, anesthesia, and CS had no effect on the polarity of the microenvironment of anular and total lipids.

4. The ERP components contributed to an increase in the number of ANS binding sites for surface proteins of erythrocytes and blood plasma. In both groups, anesthesia and CS were associated with a unilateral increase (more significantly in the ERP group) in the number of ANS binding sites for the surface charge of erythrocyte proteins and a decrease in the number of binding sites for plasma proteins.

ADDITIONAL INFO

Authors' contribution. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work. The concept and design of the study — D.R. Medzhidova, E.M. Shifman; collection and processing of material — D.R. Medzhidova, D.U. Cherkesova; statistical data processing — D.R. Medzhidova, E.M. Shifman; writing — D.R. Medzhidova, D.U. Cherkesova, A.U. Cherkesova; editing — E.M. Shifman, A.U. Cherkesova.

Funding source. This study was not supported by any external sources of funding.

Competing interests. The authors declares that there are no obvious and potential conflicts of interest associated with the publication of this article.

Ethics approval. The study was approved by the Local Ethics Committee of Dagestan State Medical University (Protocol No. 23 dated 04/17/2018).

Consent for publication. All the patients who participated in the study signed the necessary documents on voluntary informed consent to participate in the study and the publication of their medical data.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией. Концепция и дизайн исследования — Д.Р. Меджидова, Е.М. Шифман; сбор и обработка материала — Д.Р. Меджидова, Д.У. Черкесова; статистическая обработка данных — Д.Р. Меджидова, Е.М. Шифман; написание текста — Д.Р. Меджидова, Д.У. Черкесова, А.У. Черкесова; редактирование — Е.М. Шифман, А.У. Черкесова.

Финансирование. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Этическое утверждение. Проведение исследования одобрено Локальным этическим комитетом Дагестанского государственного медицинского университета (протокол № 23 от 17.04.2018).

Информированное согласие на публикацию. Все пациентки, участвовавшие в исследовании, подписали необходимые документы о добровольном информированном согласии на участие в исследовании и публикацию их медицинских данных.

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