УДК 591.111.1:546.262.3-31:57.08
https://doi.org: 10.20538/1682-0363-2020-1-94–100

Experimental estimation of the effects of exogenous carbon monoxide on blood cells

Petrova I.V.[1], Birulina J.G.[1], Trubacheva O.A.[2], Belyaeva S.N.[1], Shnaider O.L.[2], Nosarev A.V.[1], Gusakova S.V.[1], Vasilev V.N.[1], Suhanova G.A.[1]

[1] *Siberian State Medical University (SSMU)*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] *Cardiology Research Institute, Tomsk National Research Medical Center (NRMC) of Russian Academy Sciences*
111a, Kievskaya Str., Tomsk, 634012, Russian Federation

ABSTRACT

The aim of the study was to investigate the effect of the carbon monoxide (CO) donor on the Ca^{2+} -activated potassium permeability of the erythrocyte membrane and platelet aggregation ability.

Materials and methods. Healthy volunteers (n = 27) and patients with chronic coronary heart disease (CHD) (n = 32) of both sexes were examined. The material of the study was packed red blood cells and platelet-rich plasma obtained from patient's venous blood. The change of Ca^{2+} -dependent potassium conductivity of the erythrocyte membrane was evaluated by potentiometric method, and the platelet aggregation was studied by turbidimetric method. Carbon monoxide releasing molecule-2 (CORM-2) was used as a CO donor. The amplitude of A23187- and redox-induced hyperpolarization response (HR) of erythrocytes, and the rate and degree of platelet aggregation were estimated.

Results. It was shown that the addition of CORM-2 (10 and 100 μM) in the erythrocyte suspension caused a dose-dependent decrease in the amplitude of A23187- and redox-dependent HR in healthy donors, as well as in patients with chronic CHD. The maximum decrease was observed in the presence of 100 μM CORM-2. The effect of CORM-2 at concentrations of 10 and 100 μM on collagen-induced platelet aggregation led to a decrease in the degree and rate of aggregation in healthy donors. The maximum effect was shown at 100 μM of CO donor. However, such an unambiguous effect of CORM-2 on the aggregation parameters in patients with CHD was not observed.

Conclusion. The results suggest that CO has a significant effect on the ion transport function of the erythrocyte membrane and platelet aggregation activity of both healthy donors and patients with CHD.

Key words: carbon monoxide, red blood cells, ion transport systems, platelets, aggregation.

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

Source of financing. The study was funded by RFBR research project No. 18-015-00395 and by RFBR and Tomsk Region research project No. 19-415-703015.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at SSMU (Protocol No. 4340 of 30.11.2015).

For citation: Petrova I.V., Birulina J.G., Trubacheva O.A., Belyaeva S.N., Shnaider O.L., Nosarev A.V., Gusakova S.V., Vasilev V.N., Suhanova G.A. Experimental estimation of the effects of exogenous carbon monoxide on blood cells. *Bulletin of Siberian Medicine*. 2020; 19 (1): 85–93. https://doi.org: 10.20538/1682-0363-2020-1-85–93.

✉ *Petrova Irina V.*, e-mail: ivpetrova57@yandex.ru.

Экспериментальная оценка влияния экзогенного монооксида углерода на клетки крови

Петрова И.В.[1], Бирулина Ю.Г.[1], Трубачева О.А.[2], Беляева С.Н.[1], Шнайдер О.Л.[2], Носарев А.В.[1], Гусакова С.В.[1], Васильев В.Н.[1], Суханова Г.А.[1]

[1] Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

[2] Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634012, г. Томск, ул. Киевская, 111а

РЕЗЮМЕ

Цель исследования – изучить влияние донора монооксида углерода (CO) на Ca^{2+}-зависимую калиевую проницаемость мембраны эритроцитов и агрегационную способность тромбоцитов.

Материалы и методы. Обследованы здоровые добровольцы (n = 27) и пациенты с хронической ишемической болезнью сердца (ИБС) (n = 32) обоего пола. Материалом исследования являлись упакованные эритроциты и обогащенная тромбоцитами плазма, полученные из венозной крови. Потенциометрическим методом изучали изменение Ca^{2+}-зависимой калиевой проводимости мембраны эритроцитов, турбидиметрическим методом – агрегационную активность тромбоцитов при действии донора CO (CORM-2). Оценивали величину A23187- и редокс-индуцированного гиперполяризационного ответа (ГО) эритроцитов, скорость и степень агрегации тромбоцитов.

Результаты. В присутствии 10 и 100 мкМ CORM-2 амплитуда A23187- и редокс-зависимого ГО здоровых доноров, как и пациентов с хронической формой ИБС дозозависимо уменьшалась, причем максимальное снижение отмечено в присутствии 100 мкМ донора CO. Воздействие CORM-2 в концентрациях 10 и 100 мкМ на коллаген-индуцированную агрегацию тромбоцитов приводило к снижению степени и скорости агрегации у здоровых доноров, достигая максимального эффекта при 100 мкМ донора CO. Однако столь однозначного влияния CORM-2 на параметры агрегации у пациентов с ИБС не наблюдалось.

Заключение. Полученные результаты указывают, что CO оказывает существенное влияние на ион-транспортную функцию мембраны эритроцитов и агрегационную активность тромбоцитов как здоровых доноров, так и пациентов с ИБС.

Ключевые слова: монооксид углерода, эритроциты, ион-транспортные системы, тромбоциты, агрегация.

Конфликт интересов. Авторы гарантируют отсутствие потенциальных и явных конфликтов интересов, связанных с публикацией данной статьи.

Источник финансирования. Исследование выполнено при финансовой поддержке РФФИ в рамках научного проекта № 18-015-00395, РФФИ и Томской области в рамках научного проекта № 19-415-703015/19.

Соответствие принципам этики. Все участники исследования подписали добровольное информированное согласие. Исследование одобрено локальным этическим комитетом СибГМУ (протокол № 4340 от 30.11.2015).

Для цитирования: Петрова И.В., Бирулина Ю.Г., Трубачева О.А., Беляева С.Н., Шнайдер О.Л., Носарев А.В., Гусакова С.В., Васильев В.Н., Суханова Г.А. Экспериментальная оценка влияния экзогенного монооксида углерода на клетки крови. *Бюллетень сибирской медицины.* 2020; 19 (1): 94–100. https://doi.org: 10.20538/1682-0363-2020-1-94–100.

INTRODUCTION

Carbon monoxide (CO), nitric oxide (NO) and hydrogen sulfide (H2S) are a new class of gas regulatory molecules [1, 2]. The formation of CO occurs during the degradation of the hemoprotein in the heme molecule (hemoglobin, myoglobin, catalase, etc.), which is catalyzed by the heme oxygenase (HO) enzyme, that has inducible (HO-1) and constitutive (HO-2) isoforms [3].

Currently, CO is considered as an important mediator in the cardiovascular system that regulates vascular tone and has anti-inflammatory, anti-apoptotic, and antiproliferative effects [4]. There is evidence that CO modulates surface architectonics and red blood cell energy metabolism [5]. At the same time, changes in the structural and functional status of red blood cells can be an indicator of the degree of damage to membranes during various pathological processes in the body.

Abnormalities in the rheological properties of blood are of great importance among the factors determining hemodynamic disturbances in patients with coronary heart disease (CHD) [6, 7]. It has been shown that in patients with CHD, there is a change in the phospholipid composition of the erythrocyte membrane due to the increased incorporation of cholesterol, as well as possible phosphatidylserine externalization [8].

Structural disorganization and changes in the biomechanical properties of the erythrocyte membrane cause disruption in its ion transport function [9, 10], in which Gardos channels – Ca^{2+}-activated potassium channels (K_{Ca}-channels) – are significant. An increase in the activity of these channels causes eryptosis and also reduces the erythrocyte deformability. [2, 11]. A special place in the pathogenesis of CHD is given to the increased platelet aggregation and related to it relevant antiplatelet therapy. The literature data indicate that CO donors can produce an antiplatelet effect [12].

Thus, the aim of this work was to study the effect of CO on the ionic permeability of the erythrocyte membrane and platelet aggregation.

MATERIAL AND METHODS

The study included 32 patients (20 men and 12 women) aged 40 to 65 years, with a clinically verified chronic form of CHD, functional class II–III. Of these, 21 (65.6%) patients had myocardial infarction in past medical history. The patients had 5 (2; 8) years' experience of CHD. Arterial hypertension (AH) was diagnosed in 14 (43.7%) of the examined individuals. Clinical and laboratory studies were conducted before the start of conservative treatment for chronic CHD. The comparison group consisted of 27 healthy volunteers (16 men and 11 women) aged 38 to 62 years who did not have cardiovascular, endocrine or genetic diseases in the medical history. The characteristics of patient groups are given in Table 1. The work complied with the ethical standards developed in accordance with the Declaration of Helsinki (as amended in 2000) and the Guideline for good clinical practice. Each individual in this study signed a written informed consent.

Venous blood taken from elbow vein of fasted patients in the morning in test tubes of the BD Vacutainer® type with an anticoagulant served as material for the study. Hematological (XN-1000 analyzer, Sysmex, Japan), biochemical (Konelab 60i analyzer, Thermo Scientific, USA) and hemostasiological tests (coagulation analyzer ACL TOP 700, Instrumentation Laboratory Company, USA) were performed.

The erythrocyte suspension was obtained by centrifugation (5 min, 1,000 g, 4 °C) of whole blood (heparin, 17 IU / ml). Plasma and white blood cells were removed, and then the red blood cells were washed twice with 150 mM NaCl containing PBS (5 mM, pH 7,4) under the same centrifugation conditions. The erythrocyte sediment was washed with iso-osmotic medium (320 mOsm/L) containing 150 mM NaCl, 10 mM glucose, 1 mM KCl, and 1 mM $MgCl_2$. The red blood cells were transferred to ice and stored for no more than 12 hours. For the study, packed red blood cells were diluted in 1:5 ratio in their incubation medium. To obtain platelet-rich plasma, blood with sodium citrate (blood: citrate ratio – 9:1) was centrifuged for 7 min at 800 g.

The study of Ca^{2+}-activated potassium permeability of the erythrocyte membrane was conducted by the potentiometric method. The erythrocyte hyperpolarization response (HR) was estimated in response to the addition of 0.5 μM Ca^{2+}-ionophore A23187 or the ascorbate (10 mM) – phenazine methosulfate (PMS, 0,1 mM) system. The quazi stationary pH_i level was determined

during cell hemolysis in the presence of detergent Triton X-100 (0.2%).

The aggregation ability of platelets was studied by the turbidimetric method with the use of a laser analyzer (220 LA "NPF Biola", Russia). Platelet aggregation was caused by collagen at a final concentration of 2 mg/ml. The degree and rate of aggregation were estimated from the curve of the average aggregate size.

Statistical analysis of the data was performed in SPSS Statistics 17.0 using Mann – Whitney U test and Chi-square test. Data are presented as median (*Me*), interquartile range (Q_1; Q_3), and *n* (%). $p < 0.05$ was considered statistically significant.

Table 1

Clinical and laboratory characteristics of examined individuals

Parameter	Groups		*p*
	Healthy donors, *n* = 27	Patients with chronic CHD, *n* = 32	
Age, years, *Me* (Q_1; Q_3)	53 (42.5; 58)	56 (53.5; 62)	0.272
Body Mass Index, kg/m^2, *Me* (Q_1; Q_3)	24 (23; 25)	30 (28; 32)	0.028
Smoking, *n* (%)	9 (33.3)	13 (40.6)	0.041
Red blood cells, 10^{12}/л, *Me* (Q_1; Q_3)	4.6 (4.4; 4.8)	4.5 (4.3; 4.9)	0.361
Hemoglobin, г/л, *Me* (Q_1; Q_3)	147 (135; 155)	144 (131; 154)	0.118
White blood cells, 10^9/л, *Me* (Q_1; Q_3)	6.7 (5.1; 8.2)	7 (5.5; 8.4)	0.16
Platelets, 10^9/l *Me* (Q_1; Q_3)	240 (217; 264)	232 (205; 255)	0.121
INR, rel. units, *Me* (Q_1; Q_3)	1.1 (1.07; 1.14)	1 (0.95; 1.1)	0.224
aPTT, sec, *Me* (Q_1; Q_3)	30.7 (27;34.6)	28.9 (26.8;33)	0.183
Fibrinogen, g/l, *Me* (Q_1; Q_3)	3.1 (2.7; 5)	2.8 (2.5; 4.8)	0.11
Cholesterol, mmol/l, *Me* (Q_1; Q_3)	4.2 (3.6; 5)	5.5 (4.9.6.4)	0.018
Triglycerides, mmol/l, *Me* (Q_1; Q_3)	1.1 (0.6; 1.5)	2.3 (1.6; 2.7)	0.015

RESULTS

An increase in the cytosolic Ca^{2+} concentration in red blood cells, induced by the Ca^{2+}-ionophore A23187, as well as the effect of the ascorbate-PMS redox system lead to the development of a hyperpolarization response (HR). Change in the HR amplitude characterizes the conductivity of the K_{Ca}-channels of the erythrocyte membrane [11, 13].

To study the role of CO in the regulation mechanisms of erythrocyte Gardos channels, its donor, tricarbonyldichlororuthenium(II) dimer (CORM-2) - ruthenium carbonyl, was used. Despite the release of CO, which binds to hemoglobin of erythrocytes with the formation of carboxyhemoglobin (COHb), it is noted that the COHb content is less than 5%, and the effective concentration of CO is in the range of those observed in vivo [1].

It was found that under the effect of 10 and 100 μM CORM-2, the amplitudes of A23187- and redox-dependent HR of healthy donors and patients with chronic CHD decreased dose-dependently. The maximum decrease in HR was observed in the presence of 100 μM CO donor. Under the action of 10 μM CORM-2, the amplitude of Ca^{2+}-ionophore induced HR in patients' erythrocytes decreased more than that of healthy donors. For the ascorbate–PMS-caused HR this dependence was detected only in the presence of 100 μM CO donor (Table 2).

Platelet aggregation was caused by collagen, which interacts with glycoprotein VI (GPVI) and integrin $\alpha_2\beta_1$ of platelets, and, thus, triggers a complex cascade of processes, including activation of phospholipase C and phospholipase A_2, protein kinases C (PKC), MAP kinases (MAPKs), an increase in cytosolic Ca^{2+} level, thromboxane A_2 synthesis and platelet granule secretion [14, 15].

The effect of CORM-2 in concentrations of 10 and 100 μM on the collagen-caused processes in platelets led to a dose-dependent decrease in the degree and rate of aggregation in healthy donors. The maximum decrease in platelet aggregation was observed with the effect of 100 μM CO donor. However, there was no unambiguous effect of CORM-2 on the aggregation parameters in patients with coronary artery disease, although the initial values did not differ between CHD patients and healthy volunteers (Table 3).

Table 2

The effect of CORM-2 on the hyperpolarization response of erythrocytes of healthy donors and patients with chronic CHD, *Me* (Q_1; Q_3)

Parameter Group	Healthy donors, n = 27		Patients with chronic CHD, n = 32	
	Amplitude of the hyperpolarization response (HR), mV			
	A23187-induced	Redox-induced	A23187-induced	Redox-induced
Control	−25.4 (−26.3; −23.2)	−48.6 (−50.1; −47.5)	−34.7 (−37.1; −31.5) p_3 = 0.02	−49.5 (−53.7; −45.5)
+CORM-2 (10 μM)	−18.3 (−21.1; −16.9) p_1 = 0.004	−38.8 (−43.4; −34.2) p_1 = 0.003	−25.2 (−28.5; −21.4) p_1 < 0.001; p_3 = 0.004	−35.6 (−38.9; −31.2) p_1 < 0.001
+CORM-2 (100 μM)	−10.2 (−12.5; −8.4) p_1 < 0.001; p_2 = 0.01	−28.3 (−31.4; −22.7) p_1 < 0.001; p_2 = 0.003	−17.7 (−22; −13.5) p_1 < 0.001; p_2 = 0.011; p_3 = 0.02	−20.6 (−25.5; −17) p_1 < 0.001; p_2 = 0.004; p_3 = 0.005

Note. The level of statistical significance of differences in comparison with control for given HR (p_1); CORM-2 (10 μM) for given HR (p_2); the same parameter for healthy donors (p_3).

Table 3

The effect of CORM-2 on the collagen-induced platelet aggregation of healthy donors and patients with chronic CHD, *Me* (Q_1; Q_3)

Parameter Group	Healthy donors, n = 27		Patients with chronic CHD, n = 32	
	Degree of aggregation, rel. units	Rate of aggregation, rel. units/min	Degree of aggregation, rel. units	Rate of aggregation, rel. units/min
Control	10.2 (8.5; 13.2)	32.5 (29.2; 37.1)	11.8 (9.7; 13)	31.7 (26.5; 36.8)
+CORM-2 (10 μM)	5.4 (4.1; 8.2) p_1 = 0.001	22.1 (18.6; 24.5) p_1 < 0.001	10.6 (8.8; 12.1) p_3 = 0.003	22.8 (20.3; 24.4) p_1 < 0.001
+CORM-2 (100 μM)	2.3 (1.8; 3.6) p_1 < 0.001; p_2 = 0.003	10.4 (8.8; 14.3) p_1 < 0.001; p_2 < 0.001	6.1 (2.4; 5.5) p_1 = 0.008; p_2 = 0.001; p_3 = 0.015	15.6 (−21.5; −17) p_1 < 0.001; p_2 = 0.001; p_3 = 0.01

Note. The level of statistical significance of differences in comparison with control for given parameter (p_1), CORM-2 (10 μM) for given parameter (p_2); the same parameter for healthy donors (p_3).

DISCUSSION

During the past three decades, electrophysiological studies revealed that human red blood cell membrane includes a large variety of ion transporting systems that are involved in the homeostasis of cationic and, to a lesser extent, anionic cell conductivity [10]. It is known that activation of Gardos channels contributes to the release of potassium ions to the outside, causes a shift in the membrane potential towards hyperpolarization and creates a driving force for the displacement of chlorine from red blood cells. The release of cations and anions is accompanied by a loss of water, which leads to dehydration and cell shrinkage [2].

A decrease in the HR amplitude in response to the action of various concentrations of the CO donor indicates a decrease in the Ca2+-activated potassium conductivity of the membrane and leakage of potassium ions from the cell. This, probably, happens due to the interaction of CO with the channel proteins or its regulatory protein kinases [16]. At the same time, the more significant effect of CO in patients with chronic CHD, unlike healthy donors, can be associated not only with structural membrane restructuring and increased lipid peroxidation [9], but also with its antioxidant properties and increased levels of reduced glutathione (GSH) in blood cells [17]. It was found that the electron-donor system, ascorbate–PMS, promotes the formation of redox agents, which influence the Gardos channels of the erythrocyte membrane through oxidation or reduction of SH groups [18].

Platelets are small anucleate cell fragments that circulate in blood playing a crucial role in managing vascular integrity and regulating hemostasis. Nevertheless, the functional reaction of platelets can be changed either by increasing pro-aggregation stimuli or by reducing the number of antiaggregation substances. These factors contribute to increased platelet aggregation and often occur in cardiovascular diseases. It is known that CHD is associated with a systemic imbalance in hemostasis caused by the presence of a hypercoagulable state and a decrease in fibrinolysis [19]. The proportion of large platelets which are metabolically and enzymatically more active increases in patients with chronic CHD [20]. The number of platelets incapable of expressing P-selectin and having a significantly greater tendency to form microaggregates in a citrate anticoagulant increases at the same time. [21]. Larger platelets contain more prothrombotic material, with increased thromboxane A_2 and B_2 per unit volume and glycoprotein IIb–IIIa receptor expression.

In our study, it was shown that a CO donor reduced the degree and rate of collagen-induced aggregation in healthy donors and patients with CHD. A higher concentration of CO was required for the latter. Our data are consistent with the results of Chlopicki S. and colleagues [12], confirming the anti-aggregation effect of exogenous CO donors.

Also, in the presence of a guanylate cyclase inhibitor (ODQ), the decrease in collagen-induced aggregation caused by CORM-3 was not blocked, but increased. This fact indicates additional effector targets of CO in platelets. It is noted that CO, not being a powerful inhibitor of platelet activation, acquires this property when there is a lack of other antiplatelet agents (NO and prostacyclin) [12]. It is known that antiplatelet therapy, usually with aspirin, may not be effective, because there are other important ways of platelet activation that are not affected by cyclooxygenase block [22]. In this regard, CO donors appear to have high potential as antiplatelet agents.

CONCLUSION

We have found that CO has a significant effect on the ion transport function of the erythrocyte membrane and platelet aggregation in both healthy donors and patients with CHD. The CO-dependent decrease in the amplitude of Ca^{2+}- and redox-induced HR can have a positive effect in the mechanisms of regulation of erythrocyte deformability. The CO influenced decrease in platelet aggregation creates the basis for the development of methods for optimizing antiplatelet therapy in patients with CHD, in which this gasomediator participates.

REFERENCES

1. Garcia-Gallego S., Bernardes G. Carbon-Monoxide-Releasing Molecules for the Delivery of Therapeutic CO In Vivo. *Angew. Chem. Int. Ed. Engl.* 2014; 53 (37): 9712–9721. DOI: 10.1002/anie.201311225.
2. Lang E., Qadri S.M., Jilani K., Zelenak C., Lupescu A., Schleicher E., Lang F. Carbon monoxide-sensitive apoptotic death of erythrocytes. *Basic Clin. Pharmacol. Toxicol.* 2012; 111 (5): 348–355. DOI: 10.1111/j.1742-7843.2012.00915.x.
3. Ryter S.W., Choi A.M. Heme oxygenase–1/carbon monoxide: from metabolism to molecular therapy. *Am. J. Respir. Cell. Mol. Biol.* 2009; 41 (3): 251–260.
4. Motterlini R., Otterbein L.E. The therapeutic potential of carbon monoxide. *Nat Rev. Drug Discov.* 2010; 9: 728–774. DOI: 10.1038/nrd3228.
5. Tyunina O.I., Artyukhov V.G. Carbon Monoxide (CO) Modulates Surface Architectonics and Energy Metabolism of Human Blood Erythrocytes. *Bull. Exp. Biol. Med.* 2018; 165 (6): 803–807. DOI: 10.1007/s10517-018-4269-5.
6. Revin V.V., Ushakova A.A., Gromova N.V., Balykova L.A., Revina E.S., Stolyarova V.V., Stolbova T.A., Solomadin I.N., Tychkov A.Yu., Revina N.V., Imarova O.G. Study of Erythrocyte Indices, Erythrocyte Morphometric Indicators, and Oxygen-Binding Properties of Hemoglobin Hematoporphyrin Patients with Cardiovascular Diseases. *Adv. Hematol.* 2017; 2017: 8964587. DOI: 10.1155/2017/8964587.
7. Upadhyay R.K. Emerging risk biomarkers in cardiovascular diseases and disorders. *J. Lipids.* 2015; 2015: 971453. DOI: 10.1155/2015/971453.
8. Tziakas D.N., Kaski J.C., Chalikias G.K., Romero C., Fredericks S., Tentes I.K., Kortsaris A.X., Hatseras D.I., Holt D.W. Total cholesterol content of erythrocyte membranes is increased in patients with acute coronary syndrome: a new marker of clinical instability? *J. Am. Coll. Cardiol.* 2007; 49 (21): 2081–2089. DOI: 10.1016/j.jacc.2006.08.069.
9. Namazi G., Jamshidi Rad S., Attar A.M., Sarrafzadegan N., Sadeghi M., Naderi G., Pourfarzam M. Increased membrane lipid peroxidation and decreased Na+/K+-ATPase activity in erythrocytes of patients with stable coronary artery disease. *Coron. Artery Dis.* 2015; 26 (3): 239–244. DOI: 10.1097/MCA.0000000000000196.
10. Thomas S.L., Bouyer G., Cueff A., Egee S., Glogowska E., Ollivaux C. Ion channels in human red blood cell membrane: Actors or relics? *Blood Cells, Molecules & Diseases.* 2011; 46 (4): 261–265. DOI: 10.1016/j.bcmd.2011.02.007.

11. Maher A.D., Kuchel P.W. The Gardos channel: a review of the Ca^{2+}-activated K^+ channel in human erythrocytes. *Int. J. Biochem. Cell Biol.* 2003; 35 (8): 1182–1197. DOI: 10.1016/S1357-2725(02)00310-2.
12. Chlopicki S., Lomnicka M., Fedorowicz A., Grochal E., Kramkowski K., Mogielnicki A., Buczko W., Motterlini R. Inhibition of platelet aggregation by carbon monoxide-releasing molecules (CO-RMs): comparison with NO donors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 2012; 385 (6): 641–650. DOI 10.1007/s00210-012-0732-4.
13. Gusakova S.V., Kovalev I.V., Birulina Y.G., Smagliy L.V., Petrova I.V., Nosarev A.V., Orlov S.N., Aleinyk A.N. The effects of carbon monoxide and hydrogen sulfide on transmembrane ion transport. *Biophysics.* 2017; 62 (2): 220–226. DOI: 10.1134/S0006350917020099.
14. Yun S.H., Sim E.H., Goh R.Y., Park J.I., Han J.Y. Platelet Activation: The Mechanisms and Potential Biomarkers. *Biomed. Res. Int.* 2016; 2016: 9060143. DOI: 10.1155/2016/9060143.
15. Shaturny V.I., Shakhidzhanov S.S., Sveshnikova A.N., Panteleev M.A. Activators, receptors and signal transduction pathways of blood platelets. *Biochemistry (Moscow) Supplement. Series B: Biomedical Chemistry.* 2014; 60 (2): 182–200. (In Russ.).
16. Del Carlo B., Pellegrini M., Pellegrino M. Modulation of Ca^{2+}-activated K^+ channels of human erythrocytes by endogenous protein kinase C. *Biochim. Biophys. Acta.* 2003; 1612(1): 107–116. DOI: 10.1016/S0005-2736(03)00111-1.
17. Metere A., Iorio E., Scorza G., Camerini S., Casella M., Crescenzi M., Minetti M., Pietraforte D. Carbon monoxide signaling in human red blood cells: evidence for pentose phosphate pathway activation and protein deglutathionylation. *Antioxid Redox Signal.* 2014; 20 (3): 403–416.
18. Petrova I.V., Birulina Yu.G., Trybacheva O.A., Rozenbaum Yu.A., Smagliy L.V., Rydchenko V.S., Gusakova S.V. Role of sulfhydryl groups in the regulation of Ca^{2+}-activated potassium permeability of the membrane of erythrocytes in cardiovascular pathology. *Russian Journal of Physiology.* 2018; 104 (7): 827–834. (In Russ.). DOI: 10.7868/S0869813918070080.
19. Bratseth V., Pettersen A.A., Opstad T.B., Arnesen H., Seljeflot I. Markers of hypercoagulability in CAD patients. Effects of single aspirin and clopidogrel treatment. *Thromb. J.* 2012; 10 (1): 12. DOI: 10.1186/1477-9560-10-12.
20. Sharma D., Pandey M., Rishi J.P. A Study of platelet volume indices in patients of coronary artery diseases. *JSIR Journal.* 2016; 5 (5): 161–164.
21. Mcbane R.D. 2nd, Karnicki K., Tahirkheli N., Miller R.S., Owen W.G. Platelet characteristics associated with coronary artery disease. *J. Thromb.Haemost.* 2003; 1 (6): 1296–1303. DOI: 10.1046/j.1538-7836.2003.00183.x.
22. Schwartz K.A. Aspirin resistance: a clinical review focused on the most common cause, noncompliance. *Neurohospitalist.* 2011; 1 (2): 94–103. DOI: 10.1177/1941875210395776.

Authors contribution

Petrova I.V., Gusakova S.V. – critical revision for important intellectual content, approval of the manuscript for publication. Birulina J.G, Trubacheva O.A. – conception and design, interpretation and analysis of data, drafting of the manuscript. Nosarev A.V., Shnaider O.L. – manuscript substantiation. Belyaeva S.N. – experimental procedure. Vasilev V.N., Suhanova G.A. – conception and design.

Authors information

Petrova Irina V., Dr. Sci. (Biology), Professor, Department of Biophysics and Functional Diagnostics, SSMU, Tomsk. ORCID 0000-0001-9034-4226.

Birulina Julia G., Cand. Sci. (Biology), Assistant, Department of Biophysics and Functional Diagnostics, SSMU, Tomsk. ORCID 0000-0003-1237-9786.

Trubacheva Oksana A., Cand. Sci. (Med.), Researcher, Department of Functional and Laboratory Diagnostics, Cardiology Research Institute, TNRMC RAS, Tomsk. ORCID 0000-0002-1253-3352.

Belyaeva Sofia N., Student, Medical Biology Department, SSMU, Tomsk.

Shnaider Olga L., Cardiologist, Department of Atherosclerosis and Chronic Ischemic Heart Disease, Cardiology Research Institute, TNRMC RAS, Tomsk.

Nosarev Alexey V., Dr. Sci. (Med.), Professor, Department of Biophysics and Functional Diagnostics, SSMU, Tomsk. ORCID 0000-0002-0119-9707.

Gusakova Svetlana V., Dr. Sci. (Med.), Associate Professor, Head of the Department of Biophysics and Functional Diagnostics, SSMU, Tomsk. ORCID 0000-0001-5047-8668.

Vasilev Vladimir N., Dr. Sci. (Biology), Professor, Department of Physical Education and Sports, SSMU, Tomsk.

Suhanova Galina A., Dr. Sci. (Biology), Professor, Department of Biochemistry and Molecular Biology with Clinical Laboratory Diagnostics, SSMU, Tomsk.

(✉) **Petrova Irina V.**, **e-mail**: ivpetrova57@yandex.ru.

Received 24.02.2019
Accepted 25.12.2019