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HEMOSTATIC DISORDERS AND ALTERATIONS IN ERYTHROCYTE DEFORMABILITY IN EXPERIMENTAL ACUTE ISCHEMIC STROKE

Yu. Yu. Khomut, I. V. Savitsky

International University, Odesa, Ukraine

Information about authors:
Khomut Yu. Yu., **International University, Odesa, Ukraine**; e-mail: dr.khomut@gmail.com; https://orcid.org/0009-0006-6338-4488
Savitsky I. V., MD, DSc, Professor, **International University, Odesa, Ukraine**; e-mail: prof_S.I.V@ukr.net; https://orcid.org/0000-0002-5841-9993

Abstract

Background. Acute ischemic stroke is one of the leading causes of mortality and disability worldwide. Hemostatic disorders and alterations in blood rheology play a pivotal role in the pathogenesis of cerebral ischemia by promoting thrombosis, microvascular dysfunction, and impaired tissue perfusion. However, the dynamics of vascular-platelet hemostasis and erythrocyte deformability during ischemic brain injury remain insufficiently characterized.

The aim. To investigate changes in vascular-platelet hemostasis and erythrocyte deformability in rats with experimentally induced focal cerebral ischemia.

Materials and Methods. Experimental focal cerebral ischemia was induced in white non-linear rats by middle cerebral artery occlusion according to the Longa model. Platelet count was determined using the Fonio method. Platelet aggregation was assessed using ADP, adrenaline, and collagen as aggregation inducers. Erythrocyte deformability was evaluated spectrophotometrically by measuring fluctuations in optical density of erythrocyte suspensions. The parameters were assessed on days 1 and 14 after ischemia induction. Statistical analysis was performed using Student's t-test, and differences were considered significant at $p<0.05$.

Results. Experimental cerebral ischemia resulted in significant activation of the vascular-platelet component of hemostasis. On day 1, platelet count increased by 1.5-fold ($p<0.05$) compared with intact animals and remained elevated by 1.8-fold ($p<0.05$) on day 14. Platelet aggregation induced by ADP, adrenaline, and collagen increased by 1.2-, 1.3-, and 1.2-fold ($p<0.05$), respectively, during the acute phase and remained significantly elevated throughout the observation period. Assessment of erythrocyte deformability revealed marked alterations in blood rheology. On day 1, erythrocyte deformability in plasma increased by 1.4-fold ($p<0.05$), while in physiological saline it increased by 1.5-fold ($p<0.05$) compared with intact controls. By day 14, these parameters decreased slightly but remained significantly higher than control values, exceeding them by 1.2-fold and 1.3-fold ($p<0.05$), respectively.

Conclusions. Experimental focal cerebral ischemia is accompanied by persistent platelet activation, increased thrombogenic potential, and significant alterations in erythrocyte rheological properties. Enhanced platelet aggregation and changes in erythrocyte deformability contribute to microcirculatory dysfunction and may aggravate ischemic brain injury. These findings highlight the important role of hemostatic and hemorheological disturbances in the pathogenesis and progression of acute ischemic stroke.

Keywords: acute ischemic stroke; focal cerebral ischemia; hemostasis; platelet aggregation; thrombocytes; erythrocyte deformability; hemorheology; microcirculation.

Introduction. Despite advances in prevention and treatment, acute cerebrovascular accidents remain a significant medical and social challenge. Stroke is the second most common cause of death globally, responsible for 11.6% of total mortality, and ischemic stroke is the predominant subtype, representing 62.4% of all stroke cases [1, 2]. This form of brain injury leads to neuronal death and long-term disability in adults, creating a significant medical, social, and economic burden. According to current projections, one in four

individuals over the age of 25 is at risk of experiencing a stroke during their lifetime, with the probability of an IS being 18.3%. The World Health Organization predicts that between 2010 and 2050 the number of stroke cases will more than double [2].

Hemostasis plays a central role in the pathogenesis of ischemic stroke. Disturbances in vascular–platelet hemostasis, activation of the coagulation cascade, and impairment of fibrinolytic mechanisms contribute to thrombus formation within cerebral arteries, leading to reduced or complete cessation of cerebral blood flow. Endothelial dysfunction promotes platelet adhesion and aggregation, while increased levels of coagulation factors and fibrinogen enhance blood coagulability. Simultaneously, decreased fibrinolytic activity limits thrombus dissolution, facilitating vascular occlusion and the development of cerebral ischemia. Thus, an imbalance between procoagulant and anticoagulant mechanisms is a key factor in the initiation and progression of ischemic stroke [3, 4].

The aim of the study

The aim of the study was to evaluate the state of the hemostatic system, including vascular-platelet, coagulation, and fibrinolytic components, as well as erythrocyte deformability in an experimental model of acute ischemic stroke (AIS), in order to elucidate their contribution to the pathogenesis of cerebral ischemia.

Materials and methods

The study was performed on white 60 non-linear rats. Acute cerebrovascular disorder we investigated in the ischemic stroke model in rats which was reproduced using a model of endovascular occlusion of the middle cerebral artery (focal ischemia) according to E. Z. Longa. The rats were pre-anesthetised, and the surgical field was treated with a 0.05% chlorhexidine solution. After that, an incision was made in the neck area, and the common carotid artery, external carotid artery, and internal carotid artery were isolated on the right side. The common carotid artery was clamped with a vascular clip, and a No. 3 vicryl ligature was applied to the external carotid artery. The internal carotid artery was cut with scissors at a distance of 3-5 mm from the bifurcation. A 0.25 mm diameter nylon thread coated with silicone and treated with heparin solution was inserted through a segment of the internal carotid artery into the external carotid artery to a depth of 19-21 mm and fixed with a vascular clip. Blood flow was blocked for 60 minutes, after which the thread was removed. After that, the internal carotid artery was closed by coagulation until completely sealed, and the vascular clips were then removed. At the end of the operation, the incision was sutured with Vicryl No. 4 and treated with a 5% solution of brilliant green. After the operation, continuous thermometry was performed with the temperature maintained at a physiological level using

infrared lamps. During the operation, body temperature was maintained using a heating pad. The average duration of the operation was 7-10 minutes [5, 6].

Platelet count was determined using the Fonio method in stained blood smears. The method is based on counting the number of platelets per 1,000 erythrocytes, followed by calculation of the platelet concentration per microliter of blood taking into account the erythrocyte count [4].

Platelet aggregation activity was assessed by recording aggregation parameters induced by adenosine diphosphate (ADP), adrenaline, and collagen. The degree and rate of platelet aggregation were evaluated by determining the size of platelet aggregates formed during the reaction. Large aggregates became visible to the naked eye as white particles, a phenomenon known as the “snowstorm effect” [4].

Erythrocyte deformability was evaluated using a method based on the assessment of optical density fluctuations in a cell suspension. Erythrocytes were separated by centrifugation at 1,200 × g for 3 min at room temperature, followed by plasma and leukocyte removal. The erythrocyte pellet was washed three times by centrifugation under identical conditions in a chloride medium at a cell-to-medium ratio of 1:20. The hematocrit of the final suspension was adjusted to 70–74%. Erythrocyte shape and deformability were assessed by recording fluctuations in the optical density of the cell suspension during continuous stirring in a spectrophotometric cuvette using a magnetic stirrer operating at 420 rpm. Measurements were performed using an SF-4A spectrophotometer connected to a chart recorder at a wavelength of 720 nm. The optical density of the erythrocyte suspension was maintained at 0.30–0.35 units, corresponding to a hematocrit of 0.02% (approximately 3.0×10^6 cells/mL).

Disc-shaped erythrocytes scatter light anisotropically; therefore, their movement and reorientation within the suspension generate fluctuations in optical density. In contrast, spherocytes exhibit isotropic light scattering regardless of orientation and do not produce optical density fluctuations during stirring or after replacement of sulfate-containing media with chloride-containing media. Similarly, stomatocytes do not exhibit detectable optical density fluctuations. The absence of fluctuations in non-stirred suspensions confirms that the recorded changes are associated with cell reorientation within the light beam. Thus, this method allows the assessment of erythrocyte shape alterations and deformability under the influence of different media and reagents [3].

All manipulations with animals were carried out in accordance with GLP requirements, the recommendations of the State Expert Centre of the Ministry of Health of Ukraine, the General Ethical Principles of Animal Experiments (Ukraine, 2001), the Law of

Ukraine of 21 February 2006 No. 3447-IV, as amended "On the Protection of Animals from Cruel Treatment", the resolution of the First National Congress on Bioethics (Kyiv, 2007), and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [6].

Statistical analysis of the obtained results was performed using the Statistica 10.0 software package. The significance of differences between the indicators of untreated animals and experimental groups was assessed using Student's t-test and Fisher's criterion. A p-value<0.05 was considered statistically significant.

Results and discussion

The assessment of the platelet component of hemostasis revealed significant disturbances in rats with experimentally induced focal cerebral ischemia (Table 1).

On day 1 after ischemia induction, the platelet count increased by 1.5-fold ($p<0.05$) compared with intact animals, while by day 14 it was elevated by 1.8-fold ($p<0.05$). These findings indicate progressive activation of the platelet hemostatic system during the course of ischemic brain injury.

On the first day of experimental ischemia, ADP-induced platelet aggregation increased by 1.2-fold ($p<0.05$), adrenaline-induced aggregation by 1.3-fold ($p<0.05$), and collagen-induced aggregation by 1.2-fold ($p<0.05$) compared with healthy controls. By day 14, aggregation responses showed a tendency toward further elevation compared with day 1; however, these differences were not statistically significant.

Table 1

Analysis of platelet aggregation demonstrated enhanced platelet reactivity in response to various agonists (M±m)

Indicator	Intact group (n=12)	Animals with experimental focal cerebral ischemia	
		1st day (n=12)	14th day (n=12)
Platelets, 10^9/л	223,5±3,8	380,9±6,8*	400,2±7,1*
ADP-induced platelet aggregation, ($1*10^{-5}$ M), %	69,6±2,1	84,6±3,3*	80,6±3,1*
Adrenaline-induced aggregation, %	64,3±1,8	81,7±2,7*	89,4±3,6*
Collagen-induced aggregation, %	67,5±1,6	76,4±2,0*	79,8±2,2*

Notes:

1. n – number of experimental animals in each group;
2. *– p<0.05 compared to intact animals;

Nevertheless, all aggregation parameters remained significantly higher than those observed in intact animals, exceeding control values by 1.2-fold ($p<0.05$), 1.4-fold ($p<0.05$), and 1.2-fold ($p<0.05$), respectively.

The observed increase in platelet count together with enhanced aggregation activity indicates the development of a pronounced prothrombotic state during acute cerebral ischemia. Hyperactivation of platelets contributes to microvascular thrombosis, aggravates cerebral hypoperfusion, and promotes expansion of the ischemic lesion. These changes may also reflect endothelial dysfunction and increased expression of procoagulant factors, which are characteristic features of ischemic stroke pathogenesis.

Overall, the obtained results demonstrate that focal cerebral ischemia is accompanied by persistent activation of the vascular-platelet component of hemostasis, creating favorable conditions for thrombus formation and further impairment of cerebral blood flow throughout both the acute and subacute phases of ischemic injury.

One of the key pathogenetic mechanisms underlying AIS is the disruption of microcirculation and blood rheology. Increased platelet reactivity together with impaired erythrocyte deformability contributes to microvascular occlusion, aggravates tissue hypoxia, and promotes the progression of ischemic brain injury. Therefore, alongside the assessment of vascular-platelet hemostasis, the study of erythrocyte deformability provides important information regarding microcirculatory disturbances during cerebral ischemia. Normal erythrocyte deformability is essential for maintaining adequate microvascular perfusion and tissue oxygen delivery. Impairment of this property contributes to disturbances in blood rheology, hemodynamics, and overall homeostasis. In addition, spontaneous platelet aggregation associated with an increased number of circulating platelet aggregates promotes microvascular dysfunction and represents an important factor in thrombus and thromboembolism formation. Therefore, the final stage of the present study was aimed at evaluating erythrocyte deformability as a marker of microcirculatory disorders during the development of focal cerebral ischemia [7, 8, 9, 10].

Evaluation of erythrocyte deformability revealed significant hemorheological disturbances in rats with experimentally induced focal cerebral ischemia. On day 1 after ischemia induction, erythrocyte deformability in plasma increased by 1.4-fold ($p<0.05$) compared with intact animals, while the corresponding value measured in physiological saline increased by 1.5-fold ($p<0.05$). By day 14 of observation, both parameters showed a slight decrease compared with day 1, indicating partial normalization of erythrocyte rheological properties. Nevertheless, the values remained significantly elevated relative to the intact

group. Specifically, erythrocyte deformability in plasma exceeded control values by 1.2-fold (p<0.05), whereas in physiological saline it remained 1.3-fold higher (p<0.05).

These findings suggest persistent alterations in erythrocyte membrane properties and blood rheology throughout the course of cerebral ischemia. Impaired erythrocyte deformability may hinder their passage through the microvasculature, thereby compromising tissue perfusion and oxygen delivery to ischemic brain regions [3]. Together with platelet hyperactivity, these changes contribute to microcirculatory dysfunction and may exacerbate the progression of ischemic injury. Consequently, disturbances in erythrocyte deformability should be considered an important component of the pathogenesis of acute ischemic stroke and a potential target for therapeutic intervention.

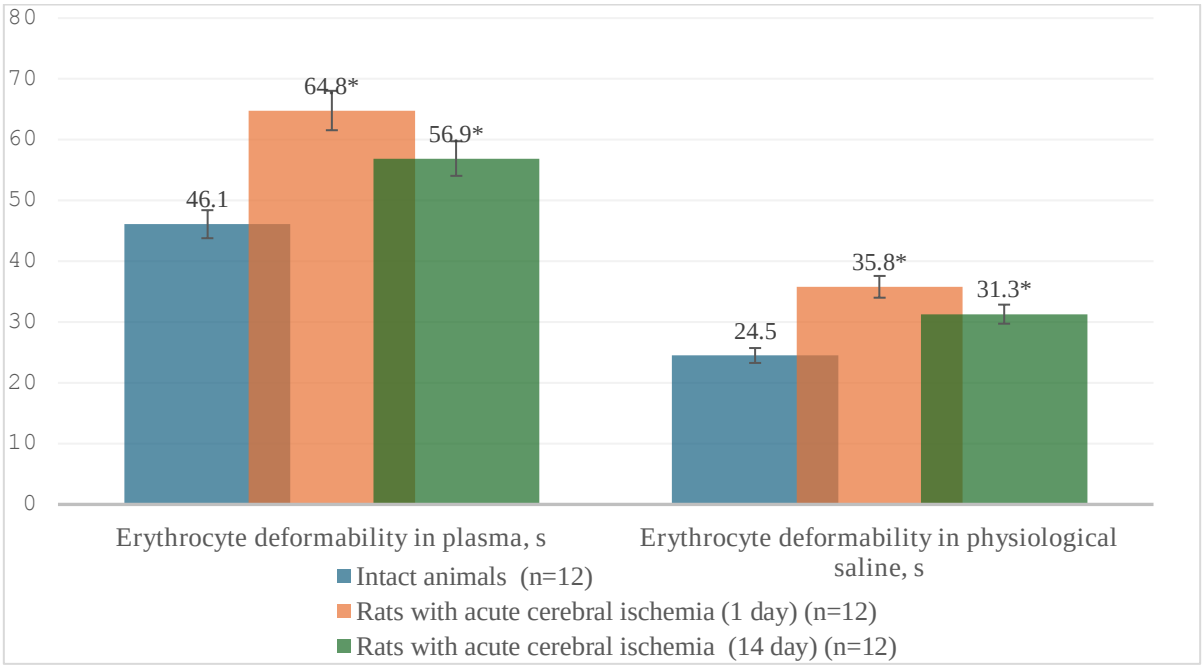

Fig. 1. Changes in erythrocyte deformability in rats with experimentally induced focal cerebral ischemia (M±m)

Notes:

3. n – number of experimental animals in each group;
4. *– p<0.05 compared to intact animals;
5. ** – p<0.05 compared to rats on day 1 of the experiment.

The obtained findings indicate the development of pronounced alterations in blood rheological properties in rats with experimental focal cerebral ischemia. The increase in erythrocyte deformability observed on the first day after ischemia induction may represent a

compensatory adaptive response aimed at maintaining microcirculatory perfusion and oxygen delivery under conditions of tissue hypoxia and impaired cerebral hemodynamics. Such changes are likely directed toward facilitating erythrocyte passage through the microvascular network and minimizing ischemic damage.

Although a slight decline in erythrocyte deformability was observed by day 14, the values remained significantly elevated compared with those of intact animals. This finding suggests persistent activation of adaptive mechanisms and ongoing hemorheological alterations during the subacute phase of ischemic injury.

The observed changes may be associated with activation of antioxidant defense systems, modifications in erythrocyte membrane architecture, alterations in membrane fluidity, and shifts in intracellular ion homeostasis. Furthermore, these adaptations may reflect a compensatory response to sustained oxidative stress and microcirculatory disturbances induced by cerebral ischemia.

Overall, the results demonstrate that experimental focal cerebral ischemia is accompanied by long-lasting disturbances in blood rheology and microcirculation, which persist throughout the observation period and may contribute to the progression of ischemic brain damage. Therefore, changes in erythrocyte deformability should be considered an important component of the pathophysiological mechanisms underlying acute ischemic stroke.

Conclusions

1. Experimental focal cerebral ischemia is accompanied by significant activation of the vascular-platelet component of hemostasis, manifested by an increase in platelet count and enhanced platelet aggregation induced by ADP, adrenaline, and collagen throughout the observation period. Persistent platelet hyperreactivity observed on days 1 and 14 after ischemia induction indicates the development of a prothrombotic state, which may contribute to microvascular occlusion and aggravation of cerebral ischemic injury.

2. Experimental cerebral ischemia induces significant alterations in erythrocyte rheological properties, evidenced by increased erythrocyte deformability in both plasma and physiological saline. These changes likely reflect compensatory adaptive mechanisms aimed at maintaining microcirculatory perfusion under ischemic conditions. Although erythrocyte deformability partially decreased by day 14, its values remained significantly elevated compared with intact animals, suggesting persistent disturbances in blood rheology and ongoing adaptation of erythrocytes to ischemia-induced hypoxia.

3. The obtained results demonstrate that acute ischemic stroke is associated with complex alterations in both hemostatic and hemorheological parameters, highlighting the important role of platelet activation and erythrocyte dysfunction in the pathogenesis and progression of cerebral ischemia.

References:

1. Saini V, Guada L, Yavagal DR. Global epidemiology of stroke and access to acute ischemic stroke interventions. *Neurology*. 2021;97(2 Suppl):6–16. doi: 10.1212/WNL.0000000000012781.

2. Muratova T, Khramtsov D, Stoyanov A, Vorokhta Y. Clinical epidemiology of ischemic stroke: global trends and regional differences. *Georgian Med News*. 2020;299:83–6. PMID: 32242851.

3. Whole Blood Viscosity and Cerebral Blood Flow in Acute Ischemic Stroke / P. Gyawali, T. P. Lillicrap, C.G. Esperon et al. *Seminars in thrombosis and hemostasis*. 2024. Vol. 50 (4). P. 580–591. https://doi.org/10.1055/s-0043-1775858

4. Platelets as mediators of neuroinflammation and thrombosis / E. Rawish, H. Nording, T. Munte, H. F. Langer. *Frontiers in immunology*. 2020. Vol. 11. e548631. https://doi.org/10.3389/fimmu.2020.548631

5. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke. 1989 Jan;20(1):84-91. doi: 10.1161/01.str.20.1.84. PMID: 2643202.

6. Reznikov O. G., Solovyov A. I., Stefanov O. V. Biotic examination of preclinical and other scientific studies performed on animals: method. *recommendations* Herald of pharmacology and pharmacy. 2006;7:47–61.

7. Endothelial dysfunction in acute ischemic stroke: a review / A. Kleeberg, T. Luft, D. Golkowski, J. C. Purrucker. *Journal of neurology*. 2025. Vol. 272 (2). e143. https://doi.org/10.1007/s00415-025-12888-6

8. Enhanced neurogenesis after ischemic stroke: The interplay between endogenous and exogenous stem cells / R. Geng, Y. Wang, R. Wang et al. *Neural regeneration research*. 2025. Vol. 21 (1). P. 212–223. https://doi.org/10.4103/NRR.NRR-D-24-00879

9. Improving Cognitive Function in Patients with Stroke: Can Computerized Training Be the Future? / R. De Luca, S. Leonardi, L. Spadaro et al. *Journal of stroke and cerebrovascular diseases.* 2018. Vol. 27 (4). P. 1055–1060. https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.11.008

10. Integrative physiological assessment of cerebral hemodynamic and metabolism

in acute ischemic stroke / J. L. Fan, R. C. Nogueira, P. Brassard et al. *Journal of cerebral blood flow and metabolism.* 2022. Vol. 42 (3). P. 454–470. https://doi.org/10.1177/0271678X211033732

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Conflict of interest.

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