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FUNCTIONAL CONDITION OF PLATELETS ON THE BACKGROUND OF STANDARD PHARMACEUTICAL TREATMENT AMONG PATIENTS WITH ISCHEMIC STROKE

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Abstract

Progression of cerebrovascular disorders after ischemic stroke (IS) remains an unresolved problem of neurology. This is largely due to the lack of knowledge about the mechanisms that modulate brain microcirculation. It is known that one of the causes of hemodynamic disorders may be the functional activity of platelets (thrombocytes), which changes in response to stimulatory signals (agonists).

The aim of the study is to determine whether it is possible to restore the functional activity of hyporeactive platelets after completion of basic pharmaceutical treatment among patients with ischemic stroke.

Material and methods. 97 patients with clinical and neuroimaging signs of cerebrovascular diseases were examined. The study includes 35 patients with a verified diagnosis of ischemic stroke of thrombotic origin. Platelets were isolated from peripheral blood by centrifugation. To stimulate platelets in vitro, ADP, Adrenaline and FAT were used in effective concentrations, which caused platelet aggregation at 50 ± 5% (EC50) among healthy individuals.

Results and discussion. 85.7% out of the 35 patients with ischemic stroke had a neurological status score of up to 10 according to the NIHSS, which is considered as a prognostically favorable factor for stroke outcome. Medicine of the 1st group (psychostimulants, nootropic drugs) were prescribed to 25 (71.4%) patients; 2nd group (drugs that have a neurotrophic effect) to all patients (100%), 3rd group (drugs for symptomatic treatment – 3rd (8.6%) and 4th group (vasoactive drugs)) to 21 (60%) patients. Medical correction of cardiovascular disorders was performed at all 35 (100%) patients with IS.

Further strategy to combat platelet dysfunction in cerebrovascular pathology will largely be based on the definition of thrombogenesis modulation processes. This approach implementation in understanding the signaling mechanisms of platelet recovery and its role in the regulation of cellular homeostasis of brain nerve tissue will not only improve the therapy prognosis, but also prevent the progression of chronic cerebral ischemia after stroke.

All human studies were conducted in compliance with the rules of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study". Informed consent was obtained from all participants.

Keywords: *ischemic stroke, cerebrovascular disorders, platelet adaptation mechanisms*

Introduction

Progression of cerebrovascular disorders after ischemic stroke (IS) remains an unresolved problem of neurology [1]. This is largely due to the lack of knowledge about the mechanisms that modulate brain microcirculation [2]. It is known that one of the causes of hemodynamic disorders may be the functional activity of platelets (thrombocytes), which changes in response to stimulatory signals (agonists) [3].

It would seem that the possibility of platelet aggregation (PA) on the background of antiplatelet therapy is minimized, despite the permanent effect of etiological factors of chronic cerebral ischemia that can stimulate platelets. These include type IV collagen of the vascular wall in the area of atherosclerotic plaque, Angiotensin II, adrenaline and serotonin, cytokines and platelet aggregation factor (PAF) [5, 6].

However, the recurrence of acute cerebrovascular accident among patients with chronic cerebral ischemia suggests the possibility of restoring the functional activity of platelets despite drug therapy. Probably, against the background of antiplatelet therapy, at patients develops adaptation of platelets aimed at restoring hemostasis, the mechanisms of which can be laid within the process of megakaryocytopoiesis (while changing the platelet phenotype), or realized / reproduced by microparticles containing RNA. At the core of these phenomena is the individual reactivity of patients characterized by genetically determined synthesis of proteins, including proteins-enzymes of intracellular signaling systems interfering / limiting the pharmacological action of medicine.

Platelet adaptation mechanisms are manifested by secretion from granules of biologically active substances (ADP, ATP, adrenaline, Ca2 +, serotonin, PAF, etc.) and are aimed at enhancing the exogenous signal (risk factor) that constantly activates platelets [7]. In the abovementioned hypothesis, there is a number of 'white spots', for example, it is not known whether the sensitivity of different receptors on platelet may differ in the selective blockade of P2Y12 receptors, α-2-adrenoceptors, angiotensin receptors type I (AT1 receptors) or blockade of TxA2 synthesis? Is it possible to classify clusters (associations) of functionally active platelet receptors? Do the clusters change during long-term pharmacological correction? Is it possible to increase the functional activity of platelets with simultaneous stimulation of several receptors in the cluster?

The aim of the study is to determine whether it is possible to restore the functional activity of hyporeactive platelets after completion of basic pharmaceutical treatment among patients with ischemic stroke.

Materials and methods

97 patients with clinical and neuroimaging signs of cerebrovascular diseases were examined. The study includes 35 patients with a verified diagnosis of ischemic stroke of thrombotic origin. Among the patients were 15 men aged 60.9 ± 2.6 (95% CI 55-68 years) and 20 women aged 69.1 ± 1.7 (95% CI 60-76 years). The stroke volume according to computed tomography was 15.5 cm3 (95% CI 7.5-37.5 cm3).

The NIHSS (National Institute of Health Stroke Scale) was used to objectify the assessment of the neurological status among patients with ischemic stroke. Depending on the number of points, the severity of the condition of patients with ischemic stroke was evaluated as follows: severe -15 points and above, moderate - 8-14 points and mild 0-7 points.

All patients with ACVA (acute cerebrovascular accident) underwent standard therapy according to the protocol aimed at optimization of external respiration; correction of cardiovascular disorders (antihypertensive therapy, diuretics, antiarrhythmic drugs); correction of hemostasis disorders; neuroprotection; correction of water-electrolyte disorders; symptomatic therapy. The study of the platelet functional state among patients was performed in 24 hours after beginning of standard pharmaceutical treatment and repeated in 9-21 days after the end of treatment at the time of hospital discharge.

Platelets were isolated from peripheral blood by centrifugation. To stimulate platelets in vitro, ADP, Adrenaline and FAT were used in effective concentrations, which caused platelet aggregation at 50 ± 5% (EC50) among healthy individuals. Platelet aggregation studies were performed on an aggregometer from ChronoLog (USA); the platelet

content in the sample was 200,000 ± 20,000 / mcl. All examined patients received an assent to participate in the study. Statistical analysis was performed using the package MedCalc 18.10.2. Point estimation of the analyzed values was performed by calculating the arithmetic mean ($\overline{X}$) or its median (Me) and the corresponding standard error (m). Student's criterion (in the case of a normal distribution law) and Wilcoxon's test (in the case of a difference in the distribution law from the normal one) were used during the analysis of intergroup differences. In all cases, the difference was considered statistically significant at a significance level <0.05.

Results

85.7% out of the 35 patients with ischemic stroke had a neurological status score of up to 10 according to the NIHSS, which is considered as a prognostically favorable factor for stroke outcome. Medicine of the 1st group (psychostimulants, nootropic drugs) were prescribed to 25 (71.4%) patients; 2nd group (drugs that have a neurotrophic effect) to all patients (100%), 3rd group (drugs for symptomatic treatment – 3rd (8.6%) and 4th group (vasoactive drugs)) to 21 (60%) patients. Medical correction of cardiovascular disorders was performed at all 35 (100%) patients with IS. In descending order of frequency of medicine prescription, it can be arranged as follows: antithrombotic (77.1%) > antihypertensive and hypolipidemic (45.7%) > diuretics (28.6%). Antiplatelet drugs were prescribed to 27 (77.1%) of the examined patients. All 27 patients took antiplatelet agents (aspirin, 150 mg / day), ie drugs aimed at platelets.

Research question – is a reliable antiplatelet effect reproduced at patients with ischemic stroke on the background of standard pharmaceutical treatment?

It was found out that after 8-14 days of treatment there was a pronounced hyporeactivity of platelets against ADP, adrenaline and PAF (Table 1), and the antiplatelet effect was reproduced in 24 hours after the appointment of acetylsalicylic acid in the dose. The data of the III quartile of the variation series concerning the ADP-induced platelet aggregation are noteworthy. In 24 h after the beginning of treatment some patients had hyperreactivity, and at the end of treatment there was platelet normoreactivity. Does this mean that using a therapeutic dose (150 mg / day) of acetylsalicylic acid, there develops cyclooxygenase (COX) resistance at patients with IS?

Analysis of patients with platelet hyperreactivity to ADP in 24 h after being placed to hospital allowed to distinguish two subgroups: A - 7 patients were not prescribed acetylsalicylic acid within 48 hours due to the need to exclude intracranial hemorrhage, B - 7 patients were prescribed this medicine at a dose of 150 mg / day, but for 24 h there did not achieve platelet hyporeactivity. Subgroup B is represented by 21 patients who were administered a drug at a dose of 150 mg / day which caused platelet hyporeactivity (Fig. 1). If we compare subgroups A and B, especially control and early results of antiplatelet therapy among patients with IS, it becomes apparent inhibition of platelet aggregation with the introduction of acetylsalicylic acid. ADP-induced platelet aggregation decreased from 75.0 ± 5.0% (95% CI 70.0-100%) to 8.0 ± 3.9% (95% CI 0-18.0%) (p <0.001). If we compare subgroups A and B, we can state about decreasing the platelet reaction to ADP by 21.3% (p <0.001) under acetylsalicylic acid influence, though the achieved effect does not reach the area of hyporeactivity, which guarantees the prevention of thrombogenesis. If we compare subgroups B and C, it should be recognized that otherwise (appointment of acetylsalicylic acid at a dose of 150 mg / day) the examined group had patients resistant to the introduction of acetylsalicylic acid at a dose of 150 mg / day.

Research question - is platelet resistant to antiplatelet therapy, including acetylsalicylic acid at a dose of 150 mg / day, at the end of hospital treatment?

The analysis of platelet group reactivity to ATC dynamics showed (Table 2) that after 8-14 days of treatment in 24 (68.6%) patients had hyporeactivity and 11 (31.4%) patients had platelet normoreactivity to ADP (reference range of normoreactivity at EC50 - 50 ± 5%). Moreover, in the 1st group after 24-48 h of treatment there revealed a standard platelet reactivity to EC50 ADP, which at the time of hospital discharge decreased by 56.1% (p = 0.021) compared to the beginning of treatment. Concerning the quartile characteristics of ADP-induced platelet aggregation, a pronounced hyporeactive effect was

achieved. The 2nd group had another trend as the platelet reactivity gradually increased (6.5 times) and reached the level of normoreactivity (95% CI 50-58%). Thus, judging by the platelet reaction to ADP, 31.4% have a platelet 'slip' from the inhibitory effect of drugs including acetylsalicylic acid at a dose of 150 mg / day. The obtained data encourage to analyze individual platelet reactivity, so it is necessary to compare the data of aggregation at the beginning and end of treatment. Transiting from the analysis of platelet group reactivity among patients with IS to the study of individual platelet reactivity to ADP, there appears possibility to investigate the specificity and severity of the agonist response, which are leveled while analyzing group reactivity; assessment of the biological significance for the body of the reproducible reaction of platelets to ADP, in particular the assessment of the platelet compensatory mechanisms; and forming an idea of the variability of the platelet sensitivity to antiplatelet therapy.

Research question - is the individual reactivity of patients with IS performed by different functional status of platelets on the background of antiplatelet therapy?

Analysis of ADP-induced platelet aggregation showed that 17 (48.6%) patients at the end of treatment had a decrease in platelet aggregation per 62.1% ($p < 0.001$), while aggregation in I and III quartiles at the beginning of treatment was 58,7% and 72%, and at the end of inpatient treatment, respectively, 8.4% and 42% (Table 3). Thus, this group of patients had an initial platelet hyperreactivity in 24-48 hours after beginning of patients' treatment, which gradually decreased to a level of platelet hyporeactivity. 15 (42.9%) patients had an increase of platelet aggregation per 7.2 times ($p < 0.001$), upon that aggregation in I and III quartiles at the beginning of treatment was 0% and 13%, and at the end of inpatient treatment respectively 30% and 56%. It is obvious that the antiplatelet effect was achieved fairly quickly and was prominent, but subsequently there was a restoration of the platelet reactivity to ADP. In 3 (8.5%) cases, when the platelet aggregation did not change by the end of treatment, platelet aggregation data in I and III quartiles were 0% and 42% at the beginning of treatment, and at the end - 5% and 40%. There is of interest the absolute number of patients who maintained platelet normo-and hyperreactivity by the end of treatment, as these can be classified as at risk of thrombogenesis. When using an ADP agonist, 13 such patients were detected (37.1%). In this group of patients, the platelet reactivity to ADP either decreased from platelet hyperreactivity to normoreactivity (5 patients, subgroup A), or increased from platelet hyporeactivity to normoreactivity (8 patients, subgroup B). In the first case, ATC decreased per 44.7% ($p = 0.063$) from 81.3 ± 11.3% up to 45.0 ± 3.7%; in the second case, it increased per 7.5 times ($p = 0.008$) from 7.35 ± 4.0% to 55.1 ± 2.1%. 22 (62.9%) patients with IS had a reliable antiplatelet effect by the end of treatment.

It can be assumed that the ADP proaggregating effect is modulated by other agonists, in particular adrenaline [8]. In fact, we are talking about the adaptive / compensatory mechanisms of platelets. It has been found that exogenous ADP involves exocytosis of endogenous ADP from dense granules (secondary wave of aggregation), which can modulate the sensitivity of purine receptors [10]. Therefore, the recorded value of ADP-induced platelet aggregation reflects the sensitivity of purine receptors and is probably the result of summing the effects of exogenous and endogenous ADP. From our point of view, the ADP secretion from dense granules and the modulation of the sensitivity of purine receptors fit into the picture of the platelet compensatory mechanisms during ischemic stroke. In this case, it is clear that ADP-induced platelet aggregation indirectly reflects the strength of the compensatory reaction, which determines the possibility of summing the effects of agonists, including adrenaline. As for the conjugation of Gi-protein stimulation of the associated signaling region by the combined action of ADP and adrenaline on platelets, it is a typical down-stream. Obviously, this mechanism implementation (in fact, also compensatory) depends on the sensitivity of purine receptors and ADP secretion.

Regarding the causes of ischemic stroke recurrence, it is logical to assume the role of neuroinflammation. If both platelet and leukocytes activity is preserved against the background of treatment of patients with chronic cerebral ischemia, preconditions are created for the recruitment of leukocytes from the vascular bed into the brain [9]. This hypothesis has not received

proper discussion in the literature, as first, the role of blood cells in achieving the desired therapeutic effect seems far-fetched, and in the progression of the disease it has not been proven. Secondly, it is a priori believed that a guaranteed reduction in the platelet reactivity is achieved already against the background of the introduction of antiplatelet agents and / or nonsteroidal anti-inflammatory drugs often added to treatment regimens. Third, platelets can be considered as important amplifiers of inflammation initiation. Thus, T. Gremmel [11] believes that the platelet interaction with neutrophils (Nf) contributes to: (a) recruitment of Nf into the site of inflammation. This interaction of Hf with platelets is mainly mediated by P-selectin, β2 and β3 integrins (CD11b / CD18, CD41 / CD61); (b) attached platelets promote secondary uptake (activation) of neutrophils and other leukocytes; (c) platelets are secreted by activators that induce the production of neutrophils cytokines and endothelial cells. So, platelets can be suggested as important amplifiers of initiation of ischemic stroke inflammation and progression.

Conclusions

Further strategy to combat platelet dysfunction in cerebrovascular pathology will largely be based on the definition of thrombogenesis modulation processes. This approach implementation in understanding the signaling mechanisms of platelet recovery and its role in the regulation of cellular homeostasis of brain nerve tissue will not only improve the therapy prognosis, but also prevent the progression of chronic cerebral ischemia after stroke.

Acknowledgments

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Table 1. Parameters of induced platelet aggregation (%) among patients with ischemic stroke on the background of standard treatment

Agonist	Median	I quartile	III quartile	Median error	Left (95%CI)	Right (95% CI)
In 24h after beginning of treatment						
ADP	28,8	6,5	66	6,606	8,4	59
Adrenaline	14	0	42	5,651	5	29,8
PAF	10	2	15	2,627	5	12
In 8-14 days of pharmaceutical treatment						
ADP	33	12,4	50	3,946	18,2	42
Adrenaline	17	7	29	3,958	10,4	27
PAF	13	5	20	2,835	7	18

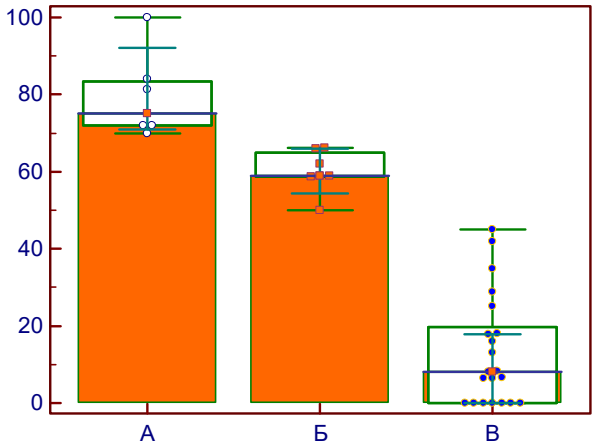

Figure 1. Parameters of ADP-induced platelet aggregation in 24 h after hospitalization of patients with ischemic stroke.
On the abscissa - subgroups A, B, B. On the ordinate – platelet aggregation (%).

Table 2. Parameters of ADP-induced platelet aggregation (%) among patients with ischemic stroke on the background of standard treatment

Time	Median	I quartile	III quartile	Median error	Left (95%CI)	Right (95% CI)
1st group (n=24)						
24 -48 h	43,5	9,7	66,0	7,5	16	62
8-14 days	19,1 p=0,021	9,2	33,5	3,4	11,4	32
2nd group (n=11)						
24-48 h	8,4	0	50	13,2	0	50
8-14 days	55 p=0,054	50	58	1,8	50	58

Table 3. Dynamics of individual platelet aggregation to ADP (%) in the medical treatment of patients with ischemic stroke

Time (days)	Median	I quartile	III quartile	Median error	Left (95%CI)	Right (95% CI)
Decrease of aggregation (n=17)						
1-2	66	58,7	72	5,383	58,7	72
8–14	25 p<0,001	8,4	42	5,381	8,4	42
Increase of aggregation (n=15)						
1-2	6,5	0	13	2,588	0	13
8–14	47 p<0,001	30	56	5,258	30	56
Platelet aggregation is not changed (n=3)						
1-2	16	0	42	15,34	0	42
8–14	12,4	5	40	13,35	5	40