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The improvement of endothelial function by inhibition of platelet activity using acetylsalicylic acid in patients with arterial hypertension



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ABSTRACT

In accordance with modern ideas about the pathogenesis of thrombotic complications of cardiovascular diseases (myocardial infarction, stroke), it should be noted that platelets and platelet humoral factors play a key role in the development of thrombosis. Activated platelets are able to activate both endotheliocytes and pro-inflammatory cells – monocytes/macrophages, which take a direct part in the formation and progression of atherosclerotic plaque.

THE PURPOSE of the study is to determine the possibility of improving the function of the endothelium by suppressing the activity of platelets using acetylsalicylic acid (ASA) in patients with arterial hypertension and atherosclerotic cardiovascular diseases.

METHODS. 41 patients with arterial hypertension and atherosclerotic cardiovascular diseases were included in the study. All patients were divided into two groups: group 1 included 20 patients who were taking ASA before the start of the study; group 2 consisted of 21 patients who did not receive ASA before participating in the study. During the study, patients of both groups received ASA (75 mg once a day) for 6 months as part of basic therapy (antihypertensives, statins). In all patients, before the start of the study and at the final stage, the activity of platelets was determined by the expression of glycoproteins GPIIb (CD41), GPIIb (CD61) and P-selectin, as well as the content in the blood of endothelial progenitor cells (CD45⁺CD31⁺CD133⁺) and desquamated endothelial cells (CD45⁺CD31⁺CD133⁺) by flow cytometry. The content of C-reactive protein, cytokines TNF- α and IL-10 and asymmetric dimethylarginine (ADMA) in the blood was determined by ELISA. All patients underwent a test with flow-dependent vasodilation of the brachial artery.

RESULTS. In patients who did not receive ASA before the start of the study, the activity of platelets in the peripheral blood was higher, signs of more pronounced endothelial dysfunction than in patients who received ASA were noted. After 6 months of taking ASA on the background of standard antihypertensive therapy, the level of activation of platelets decreased in patients of both groups. In the patients of group 1, the expression of CD41 decreased by 31.8 % ($p < 0.01$), CD61 – by 15.2 % ($p < 0.01$). In group 2, the suppression of platelet activity was even more pronounced: the level of CD41 decreased by 55.2 % ($p < 0.001$), CD61 – by 27.5 % ($p < 0.05$). In patients of group 1, the percentage of P-selectin-expressing platelets decreased by 78.1 % ($p < 0.01$); in group 2, the number of such platelets also decreased significantly – by 42.5 % ($p < 0.05$). The number of endothelial progenitors in the blood increased significantly in both groups: 3 times in patients of group 1 ($p < 0.001$); 2.3 times – in patients of group 2 ($p < 0.001$). In patients of both groups, a significant (2-fold) increase in the endothelium-dependent vasodilatation index was observed ($p < 0.01$). At the end of the study, a decrease in the blood level of CRP by 12.2 % and 18.8 %, pro-inflammatory cytokine TNF- α by 50.0 % and 57.0 %, respectively, was found in patients of groups 1 and 2 ($p < 0.001$).

CONCLUSION. The suppression of platelet activity in response to acetylsalicylic acid in patients with arterial hypertension and atherosclerotic cardiovascular diseases was accompanied by alterations in the intensity of systemic inflammation and the restoration of endothelial functions.

KEY WORDS: platelets; endothelial progenitor cells; endothelial dysfunction; arterial hypertension; acetylsalicylic acid

In accordance with modern ideas about the pathogenesis of thrombotic complications of cardiovascular diseases (myocardial infarction, stroke), it should be noted that platelets and platelet humoral factors play a key role in the development of thrombosis. It is believed that the rupture of the plaque with a violation of the integrity of the endothelial monolayer is the starting mechanism for the formation of a platelet thrombus and its first stage – platelet adhesion [1]. However, the conditions for adhesion of platelets are also created when the function of the endothelium is suppressed.

The endothelium, originally considered as a simple passive barrier, is now considered as an organ whose dysfunction is critical to the initiation, progression, and development of clinical complications of cardiovascular disease [2, 3]. The endothelium plays an important role in vascular tone, regulating thrombosis and thrombolysis, adhesion and activation of platelets to maintain uninterrupted blood flow under physiological conditions. In the conditions of intact vascular endothelium, adhesion and aggregation of platelets are prevented due to mutual electrostatic repulsion with intact endothelium as they have a negative charge, as well as due to the production of prostacyclin, nitric oxide and ecto-ADP-ase (CD39) by the endothelium [4, 5]. Under conditions of suppression of endothelial function and activation of blood platelets, the first stage of adhesion is associated with the binding of the GPIb-IX-V receptor complex of the platelet membrane to the A1 domain of immobilized VWF, which is a constitutive component of the extracellular matrix of endothelial cells [6, 7, 8]. The next stage is their aggregation. Aggregation is stimulated by agonists circulating in the blood, as well as contained in plaque and subendothelium, and released from platelets (collagen, thrombin, thromboxane A₂, platelet activation factor, serotonin, ADP, norepinephrine). Agonists stimulate the secretion of biologically active substances from platelet granules, as a result of which new platelets are included in the aggregation process. Aggregation ends with the formation of bridges between adhesive proteins (fibrinogen, von Willebrand factor) and activated glycoprotein receptors IIb/IIIa of platelets (integrin αIIbβ₃) [9, 10]. In this way, a platelet thrombus is formed, which is the basis of the pathological process. This justifies the pathogenetically based use of antiplatelet drugs in the treatment of cardiovascular diseases and for their secondary prevention [11, 12].

According to modern literature, systemic and local inflammatory processes determine the evolution of atherosclerotic plaque from the early stages of development to its rupture and atherothrombosis. There is an increasing evidence that platelets, through their complex interactions with endothelial and inflammatory cells, play an important role in initiating and maintaining this process [13, 14, 15]. Thus, circulating activated platelets can release proinflammatory and mitogenic factors in the local microenvironment, which leads to the activation of endotheliocytes and promotes the recruitment of monocytes into the subendothelium [4, 16, 17]. The initial contact between platelets and endothelial cells is mediated by P-selectin (CD62P), E-selectin and P-selectin glycoprotein ligand-1 (PSGL-1), which are expressed on the surface of endothelial cells upon their activation [18, 19, 20]. The presence of integrins, which are transmembrane receptors that mediate cell adhesion on the surface of platelets, further enhances this binding, resulting in more stable adhesion between platelets and endotheliocytes [21]. In addition, endothelial cells express ADAM-15, a member of the ADAM (disintegrin and metalloproteinase) family, a transmembrane cell surface protein that binds to platelets via the GIIb/IIIa receptor, promoting their activation. In turn, platelets enter an activated state, releasing numerous inflammatory mediators and growth factors, such as chemokines, tumor necrosis factor (TNF) superfamily, adhesion proteins, blood coagulation factors, and other mediators, which further activate endothelial cells and promote the recruitment of monocytes/macrophages to the subendothelium, which is the initial stage of atherosclerotic plaque formation [22].

Thus, activated platelets are able to activate both endotheliocytes and pro-inflammatory cells – monocytes/macrophages, which are directly involved in the formation and progression of atherosclerotic plaque, as they are the basis for the formation of foam cells [23, 24]. This interaction

occurs due to P-selectin. P-selectin, a large adhesion molecule of the selectin family, is expressed in increased concentrations on the surface of platelets during their activation, mediating interactions between endothelial cells, leukocytes, and other platelets. Activated platelets form aggregates with circulating leukocytes. The initial binding of platelets to leukocytes is mediated by the binding of P-selectin of platelets to PSGL-1 of leukocytes. After that, further activation of platelets by leukocytes occurs, and platelets contribute to the transformation of leukocytes into more adhesive and migratory forms.

In addition, the data of a number of studies indicate that platelets can affect the restoration of the endothelium by inhibiting the recruitment of endothelial progenitor cells (EPCs) at the site of vascular damage, influencing their proliferation, maturation, differentiation into cells of the endothelial phenotype and the acquisition of functional properties. Such as NO production *in vitro* [25, 26]. Such effects of platelets do not depend on direct contact between the two cell populations and are believed to be mediated through platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF).

Thus, according to a number of researchers, low-grade inflammation, endothelial dysfunction, and platelet hyperreactivity are independently associated with an increased risk of cardiovascular events. According to the results of large-scale clinical studies, inhibition of platelet activity due to long-term use of antiplatelet drugs has proven to be an effective means of reducing the risk of developing cardiovascular diseases. The prognostic benefits of ASA, Clopidogrel, and later Prasugrel and Ticagrelor were demonstrated in studies on the secondary prevention of cardiovascular diseases.

Considering the interaction between circulating platelets and vascular endotheliocytes, **the aim of the study** was to assess the potential improvement in endothelial function by inhibiting platelet activity with acetylsalicylic acid in patients with arterial hypertension and established atherosclerotic cardiovascular diseases.

MATERIALS AND METHODS

The study was performed with the informed consent of the participants in accordance with the standards of Good Clinical Practice (GCP) and the principles of the Declaration of Helsinki. The research protocol and the informed consent form were approved by the Ethics Commission of the M. D. Strazhesko National Scientific Center of Cardiology, Clinical and Regenerative Medicine NAMS of Ukraine. Examination and treatment of all patients was carried out in accordance with the current guidelines for the treatment of hypertension and cardiovascular diseases of the Ukrainian Association of Cardiology and the European Society of Cardiology [27].

41 patients with arterial hypertension and atherosclerotic cardiovascular diseases (ischemic heart disease, peripheral artery disease, ischemic stroke/transient ischemic attack) were included in the study. The average age of the patients was 61.1 ± 1.2 years; among them, 18 men (43.9 %) and 23 women (56.1 %). The study participants were divided into two groups. Group 1 included 20 patients who were constantly receiving ASA as part of the basic treatment; group 2 consisted of 21 patients who, before inclusion in the study, did not receive ASA or other antiplatelet drugs on a permanent basis. The groups were comparable regarding age and gender composition. After inclusion in the study, patients of both groups received ASA drug "Magnikor" (*Kyiv Vitamin Plant*, Ukraine) at a dose of 75 mg 1 time per day as part of the basic optimal drug therapy. The duration of observation was 6 months. At the inclusion stage and subsequently, if necessary, corrections to basic therapy (including anti-hypertensives and statins) were made.

The activity of platelets was determined in all patients at inclusion in the study and after 6 months, as well as the content of endothelial progenitor cells (EPCs) with phenotype CD45⁺CD31⁺CD133⁺ and desquamated endothelial cells (DECs) with phenotype CD45⁺CD31⁺CD133⁻ in the blood.

The functional activity of platelets was determined by the expression of glycoproteins GPIIb-IIIa and P-selectin on their surface. The determination was performed utilizing fluorochrome-labeled mouse anti-human monoclonal antibodies CD61-FITC to the GPIIb subunit, CD41-PE to the GPIIIa subunit and CD62P-PE to P-selectin (Beckman Coulter Inc., USA) on a flow cytometer NAVIOS (Beckman Coulter Inc., USA). The percentage of cells expressing corresponding surface markers, along with the relative amount of GPIIb-IIIa and P-selectin receptors on the surface of platelets measured by mean fluorescence intensity (MFI, conventional units), were estimated. For the research, peripheral blood with 3.8 % sodium citrate as an anticoagulant was diluted with phosphate buffered saline (PBS) at room temperature in a 1:100 dilution. Subsequently, 100 µL of the diluted blood was incubated for 15-20 minutes with a mixture of monoclonal antibodies to CD61 and CD41, or with a mixture of antibodies to CD61 and CD62P at room temperature, protected from light; 200 µL of PBS was added to the sample, followed by flow cytometry analysis.

To estimate EPCs and DECes by flow cytometry, 100 µL of peripheral blood with 3.8 % sodium citrate as an anticoagulant was incubated for 15-20 minutes with a mixture of mouse anti-human CD31 FITC, CD133 APC, and CD45 PC7 monoclonal antibodies (Beckman Coulter Inc., USA) at room temperature, protected from light. After the incubation, red blood cells were lysed using the OptiLyse lysing solution (Beckman Coulter Inc., USA) for 10 minutes, and 500 µL of PBS was added. To count the absolute number of cells per 1 µL, 100 µL of FlowCount fluorospheres (Beckman Coulter Inc., USA) were added to the cell suspension. The content of EPCs and DECes in 1 µL of blood was determined, along with the ratio of the number of DECes to that of EPCs (in conventional units).

A negative control (blood sample without the addition of monoclonal antibodies) and an isotype control with FITC or APC-conjugated IgG₁ and IgG_{2b} antibodies (Beckman Coulter Inc., USA) were used to set up the protocols. Flow cytometry analysis was performed on a NAVIOS flow cytometer using Navios EX Software, v. 2.2 (Beckman Coulter Inc., USA). At least 4×10⁴ cells per sample were recorded.

The kits for ELISA estimation of C-reactive protein in blood (IBL International GmbH, Germany), the pro-inflammatory cytokine TNF-α (Labor Diagnostica Nord, Germany), the anti-inflammatory cytokine IL-10 (IBL International GmbH, Germany), and the concentration of asymmetric dimethylarginine (ADMA) (Immunodiagnostik AG, Germany) were used. The data were recorded on the automatic analyzer ThunderBolt (Gold Standard Diagnostics Corp., USA) and processed using the Storm Software Suite.

To assess the dynamics of the endothelium-dependent vasodilation index in all patients at both the screening stage and after 6 months, a test involving flow-dependent vasodilation of the brachial artery was conducted.

Statistical analysis of the obtained results was carried out using methods of descriptive statistics, parametric (Student) and non-parametric (Mann-Whitney) criteria. Group comparisons were conducted using Fisher's test, and significance was set at p < 0.05 for determining differences. The data were presented as mean ± standard deviation (M ± σ).

RESULTS AND DISCUSSION

All patients included in the study had hypertension and verified cardiovascular diseases. The percentage of patients with ischemic heart disease and hypertensive kidney disease was comparable in the comparison groups (Table 1). In group 1 there were more patients with ischemic stroke/transient ischemic attack – 25.0 % vs. 14.3 % in group 2 (χ² = 4.8, p = 0.008); with heart failure – 60.0 vs. 23.8 % in group 2 (χ² = 8.3, p = 0.004) and with type 2 diabetes – 60.0 % vs. 38.1 % in group 2 (χ² = 3.4, p = 0.07). On the other hand, peripheral artery diseases occurred more often in patients of group 2 – 19.0 % vs. 5.0 % in group 1 (χ² = 5.8, p = 0.01). Cardiovascular diseases developed in all patients against the background of hypertension, the duration of which did not differ in the comparison groups.

Measurement of office blood pressure at the inclusion visit proved the ineffectiveness of the previous antihypertensive therapy, the modification of which made it possible to achieve and maintain the target blood pressure < 140/80 mm Hg in most patients. According to the anamnesis, 68.0 % of patients received statin therapy in doses that did not ensure the achievement of target levels of low-density lipoproteins. At the screening stage, all patients were prescribed high-intensity statins. According to the results of the survey conducted after 1, 3, and 6 months, patients adhered to the prescribed regime and doses of statin therapy.

Table 1. Clinical characteristics of patients at the screening stage (M ± σ).

Indicator	Group 1 (n = 20)	Group 2 (n = 21)
Duration of hypertension, years	13.1 ± 8.4	12.5 ± 7.0
Body mass index, kg/m ²	31.7 ± 3.7	32.0 ± 6.4
Diabetes, % (number of people)	60.0 (12)	38.1 (8)*
IHD, % (number of people)	70.0 (14)	71.4 (15)
Stroke, % (number of people)	25.0 (5)	14.3 (3)*
PAD, % (number of people)	5.0 (1)	19.0 (4)*
Chronic kidney disease, % (number of people)	30.0 (6)	28.6 (6)
Heart failure, % (number of people)	60.0 (12)	23.8 (5)*
Office SBP, mm Hg	160.5 ± 16.7	152.2 ± 11.4*
Office DBP, mm Hg	92.6 ± 8.6	92.4 ± 8.6

Note: * – p < 0.05 compared to group 1; IHD – ischemic heart disease; SBP – systolic blood pressure; DBP – diastolic blood pressure.

Blood platelet activity was assessed for all patients both before the commencement of the study and at its conclusion (Table 2).

Table 2. Dynamics of indicators of platelet activity and the content of EPCs and DECes of patients in the initial state and after 6 months of treatment (M ± σ).

Indicator	Group 1 (n = 20)	Group 2 (n = 21)	
MFI CD41 (GPIIb), conditional units	screening	15.1 ± 1.1	18.3 ± 1.2*
	6 months	10.3 ± 1.4**	10.1 ± 1.0**
MFI CD61 (GPIIIa), conditional units	screening	6.6 ± 0.6	9.1 ± 0.9*
	6 months	5.6 ± 0.3**	6.6 ± 0.3**
MFI CD62P (P-selectin), conditional units	screening	4.3 ± 0.3	5.8 ± 0.2*
	6 months	4.0 ± 0.5	4.4 ± 0.2
The percentage of platelets expressing CD62P, %	screening	3.2 ± 0.3	4.0 ± 0.5
	6 months	0.7 ± 0.01**	2.3 ± 0.03*
The blood content of EPCs (CD31 ⁺ CD133 ⁺), cells/µL	screening	8.0 ± 0.5	7.0 ± 0.8
	6 months	24.0 ± 4.5**	15.8 ± 1.2**
The content in the blood of DECes (CD31 ⁺ CD133 ⁺), cells/µL	screening	379.4 ± 47.4	523.9 ± 50.2*
	6 months	441.8 ± 53.7	492.5 ± 20.3
EPCs/DECes, conditional units	screening	47.4 ± 3.1	74.8 ± 5.9*
	6 months	18.4 ± 2.5**	31.2 ± 2.8**

Note: * – p < 0.05 compared to group 1; ** – p < 0.05 compared to the initial value. DECes – desquamated endothelial cells; EPCs – endothelial progenitor cells.

As can be seen from **Table 2**, at the stage of screening, a difference in the level of activation of blood platelets was noted between the groups. In patients who did not receive ASA before the start of the study (group 2), the level of platelet activity in the peripheral blood flow was higher than in patients who received ASA (group 1). Thus, patients who did not take ASA had a higher level of glycoprotein GPIIb/GPIIIa expression: by 21.2 % according to GPIIb expression ($p < 0.05$), by 37.9 % according to GPIIIa expression ($p < 0.05$). Also, patients who did not take ASA had a higher level of P-selectin (CD62P) expression by 34.9 % ($p < 0.05$). They exhibited a 25.0 % higher percentage of activated CD62P-positive platelets compared to patients who received ASA before inclusion in the study; however, the difference was not statistically significant (**Fig. 1**).

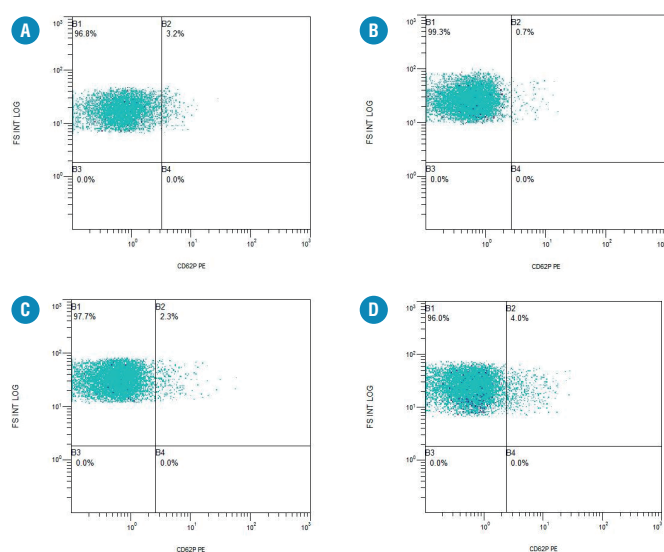


Fig. 1. Histogram of CD62P (P-selectin) expression on platelets of the studied patients according to flow cytometry data: A – patient from group 1 before treatment; B – patient from group 1 after 6 months of treatment; C – patient from group 2 before the start of treatment; D – patient from group 2 after 6 months of treatment. Quadrant B1 – platelets without of P-selectin expression (CD62P⁻); quadrant B2 – activated platelets (CD62P⁺).

The content of EPCs in the blood of patients taking ASA was 14.3 % higher than in patients who did not take ASA before the start of the study, but the difference was not reliable. The amount of DECs in 1 μ L of blood in patients who did not previously receive ASA was 38.1 % ($p < 0.001$) more than in patients who previously received ASA. Accordingly, the DECs/EPCs ratio index in group 2 was almost 2 times higher than in group 1 ($p < 0.001$). Thus, at the screening stage, patients who received ASA had a lower presence of activated platelets in the bloodstream and signs of less pronounced endothelial dysfunction than in patients who did not take ASA before inclusion in the study.

During the initial examination, both groups of patients exhibited signs of low-degree systemic inflammation. Notably, patients who took ASA before inclusion in the study showed a noticeable tendency toward a lower level of CRP (by 15 %; $p > 0.05$) and a higher level of the anti-inflammatory cytokine IL-10 (by 11 %; $p > 0.05$) compared to patients in group 2. On the other hand, the content of the pro-inflammatory cytokine TNF- α was 22.3 % ($p < 0.05$) lower in patients of group 1 (**Table 3**).

Table 3. Dynamics of indicators of systemic inflammation, endothelial function in patients at baseline and after 6 months of treatment.

Indicator	Group	Screening	6 months	p compared to the initial level
CRP, mg/L	I	4.1 \pm 1.9	3.6 \pm 1.2	0.04
	II	4.8 \pm 2.5	3.9 \pm 3.5	0.04
TNF- α , pg/mL	I	13.9 \pm 1.6	6.9 \pm 1.9*	0.001
	II	17.9 \pm 12.3	7.6 \pm 0.6*	0.001
ADMA, μ mol/L	I	0.52 \pm 0.01	0.50 \pm 0.01	n/d
	II	0.71 \pm 0.02	0.51 \pm 0.01*	0.001
Excretion of albumin with urine, mg/day	I	27.2 \pm 26.9	21.8 \pm 18.8	0.03
	II	44.7 \pm 29.1	30.9 \pm 10.4	0.045

Note: * – $p < 0.05$ compared to the initial value.

In addition, the urinary albumin excretion rate, considered as a surrogate marker of endothelial dysfunction, also tended to be lower in group 1 compared to the mean in group 2, a difference of 39.1 %, but did not reach the confidence limit. However, the blood content of ADMA in patients of group 1 was 26.8 % lower than in patients who did not take ASA before inclusion in the study ($p < 0.001$) (**Table 3**).

The assessment of the 6-month dynamics of indicators reflecting platelet activity allowed us to establish the following. The level of circulating blood platelets activation decreased in patients of both groups. Thus, in patients of group 1, the expression level of CD41 (GPIIb) decreased by 31.8 % ($p < 0.01$), CD61 (GPIIIa) – by 15.2 % ($p < 0.01$). In the group of patients who did not take ASA before inclusion in the study, inhibition of platelet activity was even more pronounced. Thus, the expression level of CD41 (GPIIb) decreased by 55.2 % ($p < 0.001$), CD61 (GPIIIa) – by 27.5 % ($p < 0.05$).

ASA also inhibited the expression of CD62P (P-selectin) on blood platelets in patients of both groups. Thus, in patients of group 1, despite a low initial level of CD62P expression, taking ASA for another 6 months was accompanied by an additional 7.0 % decrease in P-selectin expression, while in patients of group 2, this indicator decreased by 24.1 % ($p < 0.05$). There was no significant difference between these indicators in both groups after six months of treatment. The number of activated platelets expressing P-selectin significantly decreased during treatment. In patients in group 1, the percentage of platelets carrying P-selectin on the surface decreased by 78.1 % ($p < 0.01$); in group 2, the number of such platelets also decreased significantly – by 42.5 % ($p < 0.05$), but remained almost 3 times higher than in the group of patients who took ASA before inclusion in the study.

The amount of EPCs in the circulating blood increased significantly in both groups: this increase was especially more significant in patients who were taking ASA before inclusion in the study – by 3 times ($p < 0.001$). In patients of group 2, the number of EPCs increased by 2.3 times ($p < 0.001$). The number of EPCs in patients of group 1 after 6 months of taking ASA was 34.2 % higher than in patients of group 2 ($p < 0.05$). The content of DECs in the blood increased slightly in group 1 and slightly decreased in group 2 (the changes had the nature of a trend), and the difference in the amount of DECs between the groups decreased and became insignificant.

Considering the above-mentioned changes in indicators, the DECs/EPCs index in both groups significantly decreased – by 61.2 % ($p < 0.001$) in group 1 and by 58.3 % ($p < 0.001$) in group 2, but still remained smaller by 69.6 % ($p < 0.01$) in group 1 compared to group 2. The obtained data may indicate an improvement in the ability of the bone marrow to produce EPCs and a decrease in the severity of endothelial dysfunction in both groups of patients. The last statement is consistent with the dynamics of the indicator of endothelium-dependent vasodilatation. In both groups of the study, a significant increase in endothelium-dependent

vasodilatation index was observed: in group 1 from 6.6 ± 3.72 to 13.2 ± 4.1 % ($p = 0.002$), in group 2 from 6.7 ± 4.1 to 13.7 ± 2.5 % ($p = 0.001$), which reflects the positive effect of treatment on endothelial function in patients with cardiovascular diseases.

In addition, the dynamics of the plasma level of ADMA and albuminuria during observation can be signs of improvement in the functional properties of the endothelium. The concentration of ADMA decreased by 28.0 %, respectively, in patients of group 2. ASA treatment was accompanied by a probable decrease in daily albuminuria in patients of groups 1 and 2 by 19.9 % and 30.9 %, respectively, particularly among those with higher initial values. (Table 3).

According to the results of the assessment of systemic inflammation indicators during treatment, patients in group 1 and group 2 exhibited a decrease in the level of CRP in the blood by 12.2 % and 18.8 %, respectively, TNF- α level by 50.0 % and 57.0 %, respectively ($p < 0.001$), and a consistent trend toward an increase in IL-10 blood level by 20.0 % and 11.0 %, respectively (Table 3).

According to the literature, similar results were obtained regarding the effect of the use of ASA on endothelial dysfunction. Thus, in a study of 15 patients with coronary heart disease without hemodynamically significant stenosis, the number of EPCs, platelet and endothelial micropar-

ticles was determined. The latter are considered by the authors as signs of damage to the endothelium. Taking ASA 100 mg/day by patients for 8 weeks was accompanied by a decrease in the number of platelet and endothelial microparticles by 62.7 % ($p < 0.05$) and 28.4 % ($p < 0.05$), respectively, which the authors regard as a positive effect of ASA to restore the function of the endothelium. The relative number of circulating EPCs (VEGFR2⁺CD34⁺) of circulating polymorphonuclear leukocytes, remained unchanged [28].

In a prospective randomized study, the ability of P2Y₁₂-ADP receptor antagonists, capable of preventing ADP-mediated platelet activation and aggregation, to influence the number of EPCs in 106 patients with acute coronary syndrome who underwent percutaneous coronary intervention was studied. The results of the study demonstrated that Ticagrelor increases the number of EPCs in patients with acute coronary syndrome, which may promote endothelial regeneration. The authors associate this effect of the drug with its pleiotropic properties [29]. Similarly, compared with Prasugrel, Ticagrelor significantly reduced pro-inflammatory cytokines such as interleukin-6 and TNF- α and increased the number of circulating EPCs, contributing to the improvement of arterial endothelial function in 62 patients with diabetes type 2 and non-ST-segment elevation acute coronary syndrome [30].

CONCLUSION

- 1. The use of acetylsalicylic acid in patients with arterial hypertension and established atherosclerotic cardiovascular diseases was associated with the suppression of blood platelet activity, which was evident in the decreased expression of GPIIb-IIIa and P-selectin and the number of CD62P⁺ cells. These effects can lead to the suppression of platelet ability to aggregate and interact with blood cells and endotheliocytes.***
- 2. In the context of acetylsalicylic acid treatment, a statistically significant reduction in markers of systemic inflammation, including the blood level of CRP, the pro-inflammatory cytokine TNF- α , and a trend towards increased content of the anti-inflammatory cytokine IL-10, was observed.***
- 3. The observed decrease in blood platelet activation level and systemic inflammation intensity resulted in the recovery of endothelial function and enhanced bone marrow capacity to produce endothelial progenitor cells.***

REFERENCES:

1. Madhur MS, Elijovich F, Alexande M, et al. Hypertension. Do Inflammation and Immunity Hold the Key to Solving this Epidemic? *Circ Res.* 2021; 128:908-933. Available from: <http://doi.org/10.1161/CIRCRESAHA.121.318052>
2. Li Q, Youn JY, Cai H. Mechanisms and consequences of endothelial nitric oxide synthase dysfunction in hypertension. *J Hypertens.* 2015; 33:1128-1136. Available from: <http://doi.org/10.1097/HJH.0000000000000587>
3. Puzyk SG. Endothelial dysfunction in the pathogenesis of arterial hypertension and progression of atherosclerosis. *Fam Med.* 2018; 2(76):69-74. Available from: http://nbuv.gov.ua/UJRN/simmed_2018_2_14
4. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. *J Clin Invest.* 2005; 115:3378-84. Available from: <http://doi.org/10.1172/JCI27196>
5. Hamilos M, Petousis S, Parthenakis F. Interaction between platelets and endothelium: from pathophysiology to new therapeutic options. *CDT.* 2018; 8(5):568-580. Available from: <http://doi.org/10.21037/cdt.2018.07.01>
6. Boehlen F, Clemetson KJ. Platelet chemokines and their receptors: what is their relevance to platelet storage and transfusion practice? *Transfus Med.* 2001;11:403-17. Available from: <http://doi.org/10.1046/j.1365-3148.2001.00340.x>
7. Bombeli T, Schwartz BR, Harlan JM. Adhesion of activated platelets to endothelial cells: evidence for a GPIIb/IIIa-dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), alphavbeta3 integrin, and GPIbalpha. *J Exp Med.* 1998; 187:329-39. Available from: <http://doi.org/10.1084/jem.187.3.329>
8. Muttia Amalia, Meidi Utami Puteri, Fadliana Chany Saputri, Rani Sauriasari, Bambang Widyantoro. Platelet Glycoprotein-Ib (GPIb) May Serve as a Bridge between Type 2 Diabetes Mellitus (T2DM) and Atherosclerosis, Making It a Potential Target for Antiplatelet Agents in T2DM Patients. *Life.* 2023; 13(7):1473. Available from: <https://doi.org/10.3390/life13071473>
9. Zaverio M, Ruggeri, G, Loredana Mendolicchio Adhesion Mechanisms in Platelet Function *Circulation Research.* 2007; 100:1673-1685. Available from: <https://doi.org/10.1161/01.RES.0000267878.97021.ab>
10. Sun Young Cho , Mina Hur Expanded Impacts of Platelet Functions: Beyond Hemostasis and Thrombosis. *Ann Lab Med.* 2019; 39(4):343-344. Available from: <https://doi.org/10.3343/alm.2019.39.4.343>
11. Kovalenko VM, Lutai MI, Sirenko YuM, Sychoy OS. Cardiovascular diseases. Classification, standards of diagnosis and treatment. Kyiv: MORION; 2021. 320 p. [In Ukrainian]
12. Chyrchel B, Kruszelnicka O, Surdacki A. Endothelial biomarkers and platelet reactivity on ticagrelor versus clopidogrel in patients after acute coronary syndrome with and without concomitant type 2 diabetes: a preliminary observational study. *Cardiovasc Diabetol.* 2022; 21(1):249. Available from: <http://doi.org/10.1186/s12933-022-01685-4>
13. McEver RP. Adhesive interactions of leukocytes, platelets, and the vessel wall during hemostasis and inflammation. *Thromb Haemost.* 2001; 86:746-56. Available from: <http://doi.org/10.1055/s-0037-1616128>
14. Otterdal K, Smith C, Oie E, et al. Platelet-derived LIGHT induces inflammatory responses in endothelial cells and monocytes. Available from: <https://doi.org/10.1182/blood-2005-09-010629>
15. Totani L, Evangelista V. Platelet-leukocyte interactions in cardiovascular disease and beyond. *Arterioscler Thromb Vasc Biol.* 2010; 30:2357-61. Available from: <https://doi.org/10.1161/ATVBAHA.110.207480>
16. van Gils JM, Zwaginga JJ, Hordijk PL. Molecular and functional interactions among monocytes, platelets, and endothelial cells and their relevance for cardiovascular diseases. *J Leukoc Biol.* 2009; 85:195-204. Available from: <https://doi.org/10.1189/jlb.0708400>
17. Daniëlle M Coenen, Tom G Mastenbroek, Judith MEM. Cosemans. Platelet interaction with activated endothelium: mechanistic insights from microfluidics. *Blood.* 2017; 130(26):2819-2828. Available from: <https://doi.org/10.1182/blood-2017-04-780825>
18. Frenette PS, Denis CV, Weiss L, Jurk K, Subbarao S, Kehrel B, et al. P-Selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions in vivo. *J Exp Med.* 2000; 191(8):1413-22. Available from: <http://doi.org/10.1084/jem.191.8.1413>
19. Dole VS, Bergmeier W, Patten IS, Hirahashi J, Mayadas TN, Wagner DD. PSGL-1 regulates platelet P-selectin-mediated endothelial activation and shedding of P-selectin from activated platelets. *Thromb Haemost.* 2007; 98(4):806-12.
20. Braun OO, Slotta JE, Menger MD, Erlinge D, Thorlacius H. Primary and secondary capture of platelets onto inflamed femoral artery endothelium is dependent on P-selectin and PSGL-1. *Eur J Pharmacol.* 2008; 592(1-3):128-32. Available from: <http://doi.org/10.1016/j.ejphar.2008.06.102>
21. Schulz C, Schäfer A, Stolla M, et al. Chemokine fractalkine mediates leukocyte recruitment to inflammatory endothelial cells in flowing whole blood: a critical role for P-selectin expressed on activated platelets. *Circulation.* 2007; 116:764-773. Available from: <https://doi.org/10.1161/CIRCULATIONAHA.107.695189>
22. Weber C. Platelets and chemokines in atherosclerosis: partners in crime. *Circ Res.* 2005; 96:612-6. Available from: <https://doi.org/10.1161/01.RES.0000160077.17427.57>
23. Frenette PS, Johnson RC, Hynes RO. Platelets roll on stimulated endothelium in vivo: an interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci USA.* 1995; 92:7450-4. Available from: <https://doi.org/10.1073/pnas.92.16.7450>
24. Kraaijeveld AO, de Jager SC, de Jager WJ, et al. CC chemokine ligand-5 (CCL5/ RANTES) and CC chemokine ligand-18 (CCL18/PARC) are specific markers of refractory unstable angina pectoris and are transiently raised during severe ischemic symptom. *Circulation.* 2007; 116:1931-41. Available from: <https://doi.org/10.1161/CIRCULATIONAHA.107.706986>
25. Raz O, Lev DL, Battler A, et al. Pathways Mediating the Interaction between Endothelial Progenitor Cells (EPCs) and Platelets. *PLoS One.* 2014; 9:e95156. Available from: <https://doi.org/10.1371/journal.pone.0095156>
26. Karaahmet F, Kocaman S.A. Endothelial Progenitor Cells and Mesenchymal Stem Cells to Overcome Vascular Deterioration and Cytokine Storm in Critical Patients with COVID-19. *Med Hypotheses.* 2020; 144:109973. Available from: <http://doi.org/10.1016/j.mehy.2020.109973>
27. Cardiovascular diseases. Classification, standards of diagnosis and treatment / Edit. by Kovalenko VM, Lutai MI, Sirenko YM, Sychev OS. 6th edition, revised and supplemented, K.: 4th wave. 2023. 384 p. [In Ukrainian]
28. Bulut D, Becker V, Mügge A. Acetylsalicylate reduces endothelial and platelet-derived microparticles in patients with coronary artery disease. *Can J Physiol Pharmacol.* 2011; 89(4). Available from: <https://doi.org/10.1139/y11-013>

29. Bonello L, Frere C, Cointe S, Laine M, Mancini J, Thuny F, et al. Ticagrelor increases endothelial progenitor cell level compared to clopidogrel in acute coronary syndromes: A prospective randomized study. *Int J Cardiol.* 2015; 187:502-7. Available from: <http://doi.org/10.1016/j.ijcard.2015.03.414>
30. Jeong HS, Hong SJ, Cho SA, Kim JH, Cho JY, Lee SH, et al. Comparison of Ticagrelor Versus Prasugrel for Inflammation, Vascular Function, and Circulating Endothelial Progenitor Cells in Diabetic Patients With Non-ST-Segment Elevation Acute Coronary Syndrome Requiring Coronary Stenting: A Prospective, Randomized, Crossover Trial. *JACC Cardiovasc Interv.* 2017; 10(16):1646-1658. Available from: <http://doi.org/10.1016/j.jcin.2017.05.064>



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Покращення функції ендотелію шляхом пригнічення активності тромбоцитів за допомогою ацетилсаліцилової кислоти у хворих з артеріальною гіпертензією



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РЕЗЮМЕ

Згідно з сучасними уявленнями щодо патогенезу тромботичних ускладнень серцево-судинних захворювань (інфаркту міокарда, інсульту), слід зазначити, що ключова роль у розвитку тромбозу належить тромбоцитам і тромбоцитарним гуморальним факторам. Активовані тромбоцити спроможні активувати як ендотеліоцити, так і прозапальні клітини – моноцити/макрофаги, що беруть безпосередню участь у формуванні та прогресуванні атеросклеротичної бляшки.

МЕТА РОБОТИ – визначити можливість покращення функції ендотелію шляхом пригнічення активності тромбоцитів за допомогою ацетилсаліцилової кислоти (АСК), у хворих на артеріальну гіпертензію та атеросклеротичні серцево-судинні захворювання.

МЕТОДИ. В дослідження був включений 41 пацієнт з артеріальною гіпертензією та встановленими атеросклеротичними серцево-судинними захворюваннями. Всі пацієнти були розподілені на дві групи. До групи 1 увійшло 20 пацієнтів, які до початку дослідження приймали АСК; групу 2 склали 21 пацієнт, які до участі в дослідженні не отримували АСК. Під час дослідження пацієнти обох груп отримували АСК (75 мг 1 раз на добу) протягом 6 місяців у складі базисної терапії (антигіпертензивні, статини). Всім пацієнтам до початку дослідження та на завершальному етапі проводили визначення активності тромбоцитів за експресією на їх поверхні глікопротеїнів GPIIb-IIIa та P-селектину, а також вмісту в крові клітин-попередників ендотеліоцитів (фенотип CD45-CD31⁺CD133⁺) та злущених ендотеліальних клітин (фенотип CD45-CD31⁺CD133⁺) методом проточної цитометрії. Вміст в крові С-реактивного білка, цитокінів TNF- α та IL-10 і асиметричного диметиларгініну (ADMA) визначали імуноферментним методом. Всім пацієнтам проводили пробу з потік-залежною вазодилатацією плечової артерії.

РЕЗУЛЬТАТИ. У пацієнтів, які до початку дослідження не отримували АСК, рівень активності тромбоцитів в периферійному кровотоку був вищим, відмічались ознаки більш вираженої дисфункції ендотелію ніж у пацієнтів, які її приймали. Через 6 місяців прийому АСК на фоні стандартної антигіпертензивної терапії рівень активації циркулюючих тромбоцитів крові зменшився у пацієнтів обох груп. У пацієнтів 1 групи рівень експресії CD41 (GPIIb) зменшився на 31,8 % ($p < 0,01$), CD61 (GPIIIa) – на 15,2 % ($p < 0,01$). У пацієнтів групи 2 пригнічення активності тромбоцитів було ще більш вираженим: рівень експресії CD41 (GPIIb) зменшився на 55,2 % ($p < 0,001$), CD61 (GPIIIa) – на 27,5 % ($p < 0,05$). У пацієнтів групи 1 відсоток тромбоцитів, які несли на поверхні P-селектин, знизився на 78,1 % ($p < 0,01$); в групі 2 кількість таких тромбоцитів також суттєво знизилась – на 42,5 % ($p < 0,05$). Кількість клітин-попередників ендотеліоцитів в циркулюючій крові суттєво зросла в обох групах: у 3 рази у пацієнтів групи 1 ($p < 0,001$); у 2,3 рази – у пацієнтів групи 2 ($p < 0,001$). У пацієнтів обох груп спостерігали суттєве (в 2 рази) збільшення індексу ендотеліозалежної вазодилатації ($p < 0,01$). Наприкінці дослідження виявлено зменшення рівня в крові С-реактивного білка на 12,2 % і 18,8 %, прозапального цитокіну TNF- α – на 50,0 % і 57,0 % відповідно у пацієнтів групи 1 та 2 ($p < 0,001$).

ВИСНОВОК. Пригнічення активності тромбоцитів крові за допомогою ацетилсаліцилової кислоти у пацієнтів з артеріальною гіпертензією та встановленими атеросклеротичними серцево-судинними захворюваннями супроводжувалось зменшенням інтенсивності системного запалення та відновленням функції ендотелію.

Ключові слова: тромбоцити; клітини-попередники ендотеліоцитів; ендотеліальна дисфункція; артеріальна гіпертензія; ацетилсаліцилова кислота