Original Article

Evaluation Of Platelet Aggregation Activity Depending on The Duration of Antiplatelet Administration in Patients with Coronary Heart Disease

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Abstract

To study the aggregation activity of platelets in patients with ischemic heart disease depending on the long-term intake of acetylsalicylic acid (ASA). Material and methods. The study included: 116 patients with coronary artery disease with stable angina pectoris, taking a standard dose of aspirin 75 mg/day. during various terms. Patients with SSN were divided into 4 groups according to the duration of taking ASA. Platelet aggregation was measured using a laser platelet aggregation analyzer Alat-2 Biol, with computer processing according to the AGGR program. Adenosine diphosphate (ADP) in concentrations of 0.1, 1.0, 5.0 mmol/l was used as an inductor. The results of the study of patients taking ASA for more than 5 years showed that spontaneous aggregation and ADP-induced platelet aggregation were higher than in patients taking the drug for less than 5 years. Statistically significant differences were observed when compared with a group of patients taking ASA for less than a year (spontaneous aggregation of 1.7±0.09 ($p<0.05$), 0.1 μm ADP-induced aggregation of 6.58±0.86 ($p<0.05$), 1.0 μm ADP-induced aggregation of 16.51±1.63 ($p<0.01$), 5.0 μm ADP-induced aggregation 58.44±3.72 ($p<0.01$). Conclusion. Thus, the results showed that with an increase in the duration of aspirin intake, there was a change in both spontaneous and ADP-induced platelet aggregation with a statistically significant increase.

Keywords: acetylsalicylic acid; coronary heart disease; spontaneous platelet aggregation, ADP-induced platelet aggregation, aspirin resistance.

INTRODUCTION

Coronary heart disease (CHD) is one of the most important social problems of the healthcare system and is considered one of the leading causes of mortality and disability among the world's population. In particular, due to the development of acute ischemia as a result of atherothrombosis. Disorders of platelet functional activity play a leading role in the pathogenesis of one of the main clinical forms of coronary heart disease - angina pectoris, which determines the relevance of studying various mechanisms of their adhesion and aggregation. External signs of platelet activation are a change in shape, an increase in the secretion of biologically active substances from granules, increased aggregation (AT). The direct activators of triggering these processes can be an increase in the shear rate, thrombin, adenosine diphosphate (ADP), collagen, biogenic amines, which also cause the release of the most powerful aggregation activator - thromboxane A2 (THA2) [4].

One of the drug-based approaches to the prevention of thrombosis and the development of acute ischemia in patients with coronary heart disease is antiplatelet therapy. Currently, it has been proven that the appointment of antiplatelet drugs for primary and secondary prevention of acute cardiovascular events is a mandatory component of the pharmacotherapy of coronary heart disease and is included in the clinical recommendations of different levels [5, 6, 7,]. Acetylsalicylic acid (ASA, aspirin), whose clinical efficacy has been proven by numerous clinical studies, remains the most common and affordable drug that inhibits platelet aggregation [2, 11].

However, in clinical practice, after regular intake of aspirin by patients, thrombosis phenomena still occur [1,9,14]. This phenomenon is known as aspirin resistance. In recent years, the amount of evidence confirming a high risk of atherothrombosis has been increasing in individuals who have a low reaction to antithrombotic drugs. In this category of

patients, there are cases of cardiovascular events - myocardial infarction (MI), acute disorders of cerebral circulation, sudden death [15, 16, 17]. In large multicenter studies, such as HOPE (The Heart Outcomes Prevention Evaluation study Investigators, 2000), PLATO (A Platelet Inhibition and Patient Outcomes, 2010), combined ROC analysis (combined receiver operator curve, 2013), it was found that insufficient inhibition of platelet functional activity by antiplatelet drugs is one of the leading causes of mortality, development of myocardial infarction and stent thrombosis [20].

Currently, aspirin resistance is usually determined when the expected effect of platelet anti-aggregation does not manifest itself after patients regularly take the usual dose of aspirin, and laboratory indicators show that the activity or rate of platelet accumulation is not ideal, which leads to an increased risk of cardiovascular events [13]. Henry et al. [12] evaluated the peak and minimum biological efficacy of 2 to 24 hours of daily low-dose aspirin intake in 150 patients with stable coronary artery disease. The light transmission concentration (LTA) induced by 0.5 mg/ml of arachidonic acid was measured. It was found that aspirin resistance appeared in a quarter of patients. A study conducted on 126 Asian-Indian patients with stable coronary artery disease showed that 36% of patients had no response to aspirin [9]. In a systematic review and meta-analysis of 65 studies involving 10,729 patients, the overall prevalence of AR in patients with CVD, determined in the laboratory, was 24.7% (95% CI 21.4–28.4). Its risk was higher in women than in men, with a coefficient of 1.16 (95% CI 0.87–1.54) [10]. These data indicate that AR is a frequent clinical phenomenon and may affect therapeutic efficacy.

The aim of this study was to evaluate the aggregation activity of platelets in patients with coronary heart disease who took aspirin for a long time.

MATERIALS AND METHODS

The study included 116 patients who were admitted to the cardiology department of the Republican Specialized Scientific and Practical Medical Center for Therapy and Medical Rehabilitation with a diagnosis of coronary heart disease: stable angina pectoris (SSN) of II or III functional classes (FC). The diagnosis of coronary heart disease was established in accordance with the criteria of the European Society of Cardiology [13, 21]. Patients with SSN were divided anamnetically into 4 groups according to the prescription of ASA. Group 1 consisted of 30 patients who took aspirin up to 1 year, the second group consisted of 31 patients who took aspirin from 1 year to 5 years, the third group consisted of 31 patients who took aspirin for more than 5 years and the control group consisted of patients who did not receive aspirin. The average age of patients was 64.9±0.9 years, among the patients there were 55.1% (63 patients) men, 44.8% (53 patients) women.

Table 1 presents the characteristics of patients with coronary heart disease.

Table (1): Clinical and anamnestic characteristics of patients with coronary heart disease

Indicators	**Number of patients (n=116)**			
	Group 1 – I accept up to 1 year current ASK (n=30)	2-group – From 1 to 5 years I accept current ASK (n=31)	3-group – I have been taking over 5 years current ASK (n=31)	Control group Patients not receiving ASA (n=24)
Age, years	60,4±1,68	64,6±1,6	63,0±1,34	60,8±0,69
Paul				
	абс.(%)	абс.(%)	абс.(%)	абс.(%) %
Men, people	15 (50)	17 (5,8)	18 (58)	13 (54,3)
Women, people	15 (50)	14 (45,2)	13 (42)	11 (45,8)
BMI				
Obesity (BMI30)	12 (40)	8 (25,8)	13 (41,9)	8 (33,3)
Bad habits				
Smoking	11 (36,7)	12 (38,7)	11 (35,5)	8 (33,3)
The main disease				
Stable angina pectoris of tension II FC	22 (73,3)	21 (67,7)	24 (77,4)	20 (64,5)

Stable angina pectoris of tension III FC	8 (10)	10 (32,3)	7 (22,6)	11 (35,5)
Postinfarction cardiosclerosis more than a year ago	8 (26,7)	8 (25,8)	8 (25,8)	6 (24)
Complications				
CHF with preserved systolic function (LV>45-55%)	15 (50)	13 (41,9)	19 (61,2)	10 (41,6)
Concomitant diseases				
Hypertension II art .	20 (66,7)	21 (67,1)	24 (77,4)	16 (66,7)
Type II diabetes mellitus	12 (40)	8 (25,8)	13 (41,9)	8 (33,3)
Laboratory data				
	M±m	M±m	M±m	M±m
Red blood cells	4,44±0,17	4,43±0,12	4,43±0,09	4,36±0,09
Platelets	207±13,03	213±11,9	218±12,2	211±8,2
Cholesterol	4,9±0,2	5,02±0,23	4,7±0,14	4,54±0,2
LDL	2,±0,27	1,52±0,11	1,66±0,12	1,7±0,15
LDL	1,03±0,05	1,10±0,05	0,98±0,02	1,02±0,04
HDL	3,55±0,16	3,55±0,19	3,26±0,12	3,19±0,16
Triglycerides	0,38±0,05	0,29±0,02	0,33±0,02	0,34±0,30

Note: BMI– body mass index**; CHF –** chronic heart failure**, PV –** ejection fraction**, LDL-**low density lipoproteins, **VLDL-**very low density lipoproteins**, HDL-**high density lipoproteins**.**

All patients received cardioselective beta-blockers, ACE inhibitors or sartans and statins, which corresponds to the standard therapy of coronary heart disease. According to the anamnesis, all patients except the control group received 75 mg of ASA as an antiplatelet.

All patients underwent conventional methods of examination (clinical and biochemical blood tests, ECG, EchoCG,).

Platelet aggregation in platelet-rich citrate plasma was studied using the Born [8] and O'Brien method on a two-channel laser platelet aggregation analyzer "Alat-2 Biola" (Russia), with computer processing according to the AGGR program. In this device, platelet aggregation is checked by the usual turbodimetric method, which registers changes in the light transmission of platelet-enriched serum (SOT). Adenosine diphosphate (ADP) solutions with final concentrations of 0.1, 1.0 and 5 micrograms/ml were used as aggregation inducers. The norm was taken as: for spontaneous aggregation - 1.0–1.5 rel.units, for induced 0.1 mmol of ADP - 1.0–2.0 rel.units, for induced 1.0 mmol of ADP - 1.5–5.5 rel. units and for induced 5 mmol of ADP - 25-72%. The types (one- or two-wave) and reversibility (reversible or irreversible) of aggregation curves in patients with and without aspirin resistance were also determined.

Statistical data processing was carried out using the SPSS 18.0 software.

RESULTS AND DISCUSSIONS

The study showed that all indicators of platelet aggregation were statistically significantly higher in patients who did not receive ASA in the control group, compared with patients of groups 1 and 2 who received ASA ($p < 0.05$). It was also found that aggregation rates were lower in group 3 of patients with a duration of ASA administration of more than 5 years compared with the control group, but the differences were not statistically significant ($p > 0.05$).

From the data presented in Tables 2 and 3, it can be seen that there are differences between groups with different duration of aspirin intake in spontaneous and ADP-induced platelet aggregation. In particular, the aggregation rates of the 1st group were the lowest compared to the 2nd and 3rd groups.

Table (2): Parameters of platelet aggregation in patients with coronary heart disease depending on the duration of aspirin intake, M±SD

Aggregation parameters, rel.units	Control group Patients not receiving ASA	Group 1 – I accept up to 1 year current	2-group – From 1 to 5	3-group – I have been taking over 5

	(n=24)	ASK (n=30)	years I accept current ASK (n=31)	years current ASK (n=31)
Spontaneous aggregation, rel.units	1,96±0,1	1,44±0,09 P<0,001	1,54±0,1 P<0,01 P1>0,05	1,7±0,09 P>0,05 P1<0,05 P2>0,05
0.1 microns of ADP, rel.units	8,1±0,98	4,37±0,73 P<0,01	6,06±0,79 P<0,05 P1>0,05	6,58±0,86 P>0,05 P1<0,05 P2>0,05
1.0 microns of ADF, rel.units	18,97±1,66	10,73±1,33 P<0,001	14,67±1,6 P<0,05 P1>0,05	16,51±1,63 P>0,05 P1<0,01 P2>0,05

Note: p - compared with the indicators of the control group**,**

p1 - compared with the indicators of the 1st group**,**

p2 - compared with the indicators of the 2nd group.

Table (2): Parameters of 5.0 μm of ADP-induced platelet aggregation in patients with coronary heart disease, depending on the duration of aspirin intake, M ± SD

Aggregation Parameters, %	Control group Patients not receiving ASA (n=24)	Group 1 – I accept up to 1 year current ASK (n=30)	2-group – From 1 to 5 years I accept current ASK (n=31)	3-group – I have been taking over 5 years current ASK (n=31)
5.0 microns of ADF, %	61,33±3,71	43,18±3,56 P<0,001	49,72±3,77 P<0,05 P1>0,05	58,44±3,72 P>0,05 P1<0,01 P2>0,05

Note: p - compared with the indicators of the control group**,**

p1 - compared with the indicators of the 1st group**,**

p2 - compared with the indicators of the 2nd group.

In patients of group 3, the indicators of spontaneous aggregation and ADP-induced platelet aggregation were higher than in patients of groups 1 and 2, and statistically significant differences were observed compared with patients of group 1 (in group 1, spontaneous aggregation was 1.44±0.09 $p<0.05$, 0.1 microns of ADP-induced aggregation 4.37±0.73 $p1<0.05$, 1.0 μm ADP-induced aggregation 10.73±1.33 $p1<0.01$, 5.0 μm ADP-induced aggregation 43.18±3.56%, in group 3 spontaneous aggregation 1.7±0.09 $p<0.05$, 0.1 μm ADP-induced aggregation 6.58±0.86 $p1<0.05$, 1.0 μm ADP-induced aggregation 16.51±1.63 $p1<0.01$, 5.0 μm ADP-induced aggregation 58.44±3.72%, respectively, $p1<0.01$). It should be noted that there was no significant difference in platelet aggregation in patients of group 2 compared to patients of groups 1 and 3. Thus, the results obtained showed that with an increase in the duration of aspirin intake, there was a change in both spontaneous and ADP-induced platelet aggregation with a statistically significant increase ($p<0.05$).

In patients with increased platelet aggregation, changes were observed more often with spontaneous and induced platelet aggregation at a concentration of 5.0 mmol ADP. It was also found that in patients, an increase in spontaneous and 5.0 mmol ADP-induced platelet aggregation was often associated with irreversible and single-wave types of aggregation curves. Graphical curves of spontaneous and 5.0 mmol ADP induced aggregation of patients are shown in Figures 1 and 2.

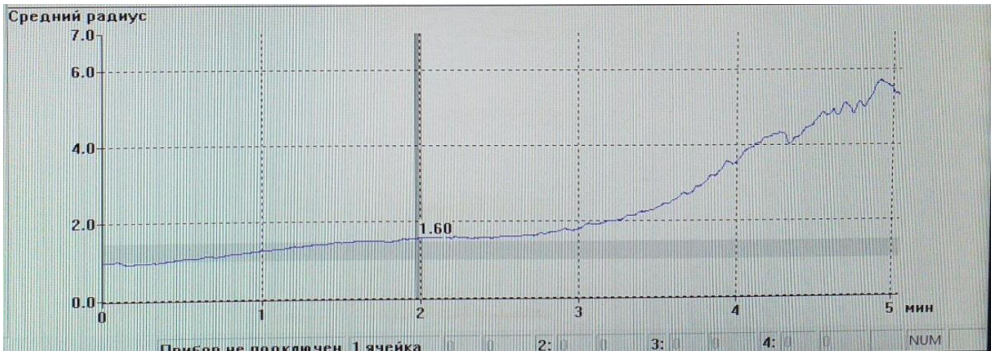

Figure (1): Spontaneous platelet aggregation: R-amplitude of the curvature of 1.6 rel..ed. The type of curves is single-wave, irreversible.

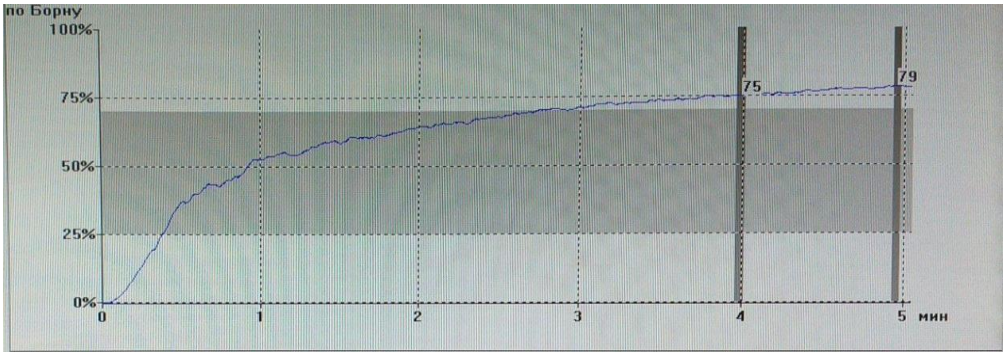

Figure (2): Platelet aggregation induced by 5.0 ADP: the curvature amplitude was 79%, which is associated with the fusion of the release phase with the induced aggregation phase. The type of curves is single-wave, irreversible**.**

Thus, based on the results of the conducted studies, it can be said that with prolonged use of ASA (more than 5 years), there is an increase in the aggregation ability of platelets in both spontaneous and ADP-induced forms ($p<0.05$).

DISCUSSION

From the point of view of evidence-based medicine, the use of antiplatelet drugs for the purpose of primary and secondary prevention of cardiovascular diseases is the main component of the pharmacotherapy of coronary heart disease. The use of antiplatelet drugs leads to the cessation of the induction of aggregation in the remodeling processes in the cardiovascular system due to a decrease in the functional activity of platelets. In recent years, there has been a growing body of evidence confirming the occurrence of insufficient inhibition of platelet aggregation in patients who have been taking aspirin in small doses for a long time. According to P.P. Ogursov and co-authors [4], an increase in the number of patients who had hyperagregation was associated with the duration of aspirin intake and a high degree of approximation reliability ($R2 = 0.9063$). The prognosis of patients with hyperagregation taking aspirin is 70% after 2 years. According to the results of our study, changes in platelet aggregation, with an increase in spontaneous and 5.0 mmol ADP-induced platelet aggregation in the groups receiving and not receiving ASA in patients with coronary heart

disease SSN II-III FC, were observed more often and led to a statistically significant increase in both spontaneous and ADP-induced aggregation characteristics of platelets. A significant aspect was that when taking aspirin for a longer period (especially for a period of more than 5 years), compared with patients who received aspirin for a shorter period of time, platelet aggregation ability led to an increase in both spontaneous and 5.0 μm ADP-induced platelet aggregation ($p<0.05$).

According to the pathophysiological mechanism, the state of aggregation resistance to aspirin, which appears against the background of prolonged use of ASA, may be either conformational or associated with a change in the cyclooxygenase enzyme during thrombocytopoiesis, which may lead to a violation of the interaction of this enzyme with ASA. According to L.I. Buryachkovsky and co-authors (2011), a decrease in the aggregation of induced platelets is associated with a greater antiplatelet effect on ADP-induced aggregation and a lesser effect on spontaneous aggregation [3]. Also, if platelets are strongly stimulated for a long time, their irreversible activity occurs. In this situation, platelets accumulate in other cells or extracellular structures, general degradation occurs and the secretion of internal contents occurs. If there is a mass appearance of platelets with irreversible activity, a significant decrease is observed in all inducers. Microscopy records a large number of deformed platelets [18]. But this fact, that is, the presence of a large amount of hyperagregation in patients against the background of taking the drug ASA, indicates a low level of thrombogenic potential in patients. ADP-induced aggregated thrombocytosis can lead to myocardial infarction and occlusion, and the indicator of spontaneous platelet aggregation can again be a reliable marker of myocardial infarction and the risk of occlusion [19, 22].

According to the results of the study, a spontaneous or ADP-induced increase in platelet aggregation in patients treated with ASA was found in almost every individual patient in the study. The fact that spontaneous hyperreactivity also persists in patients receiving ASA indicates that the risk of thrombosis increases.

CONCLUSIONS

1. With an increase in the duration of aspirin intake, there was a change in both spontaneous and ADP-induced platelet aggregation with a statistically significant increase.

2. In patients with increased platelet aggregation, changes in spontaneous aggregation and when exposed to an inducer at a concentration of 5.0 mmol were most often recorded, both against the background of taking ASA and without it.

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