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Molecular and metabolic characteristics of changes in the platelet sensitivity to antiplatelet therapy in patients with coronary artery disease before and after coronary artery bypass grafting

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Aim. To study the production of reactive oxygen species (ROS) by platelets in patients with coronary artery disease (CAD) before and after coronary artery bypass grafting (CABG), depending on their sensitivity to acetylsalicylic acid (ASA) as a part of ASA monotherapy and dual antiplatelet therapy (DAPT) (ASA+clopidogrel).

Material and methods. The study included 104 patients with CAD (ASA monotherapy, 64 patients; DAPT, 40 patients). From day 1 after CABG, they took 100 mg a day of enteric-coated ASA. In the DAPT group, clopidogrel was prescribed for 2-3 days after CABG. All measurements were performed before surgery, on the 1st day and days 8-10 after surgery. Control group consisted of 36 healthy donors. Resistance to ASA was determined at a level of optical platelet aggregation with arachidonic acid ≥20% at least at one observation point. The spontaneous and ADP-induced chemiluminescence (CL) of platelets with luminol and lucigenin was assessed according to the following parameters: time to maximum intensity (Tmax), maximum intensity (Imax), area (S) under the CL curve, and the ratio of ADP-induced CL S to spontaneous CL S.

Results. Throughout the study, 71 patients with CAD were sensitive to ASA (sASA) (ASA monotherapy, 46 patients; DAPT, 25 patients), three patients — resistant (rASA) (ASA monotherapy, 1; DAPT, 2). Sensitivity of other 30 patients (ASA monotherapy, 17; DAPT, 13) changed in different follow-up periods. Compared to the control group, sASA patients had increased values of platelet CL parameters throughout the study, while in the rASA group (ASA monotherapy), Tmax was higher before CABG, and in the rASA group (ASA therapy+clopidogrel), Imax and S were higher on the first day after CABG, while Imax — on days 8-10 after CABG. Compared to sASA, the values of S and Imax before CABG, Imax after CABG, as well as Imax and S on the days 8-10 after CABG in rASA (ASA monotherapy) were significantly lower, while in rASA (ASA therapy+clopidogrel), only the Tmax values were lower on the 8-10 days after CABG.

Conclusion. In patients with CAD, depending on the sensitivity to ASA and antiplatelet therapy after CABG, the metabolic activity of platelets in terms of ROS production differs. In sASA patients, ROS synthesis is higher than in healthy individuals, while, in rASA patients (ASA monotherapy), platelets produce ROS levels lower than in sASA. CABG surgery and the addition of clopidogrel to ASA therapy leads to increased ROS production in rASA patients in the postoperative period.

Keywords: coronary artery disease, clopidogrel, acetylsalicylic acid, resistance, reactive oxygen species, platelet.

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Cardiovascular disease is a leader among all causes of death, affecting the population decline, including working-age persons, which is a socially significant problem that requires close attention and search for solutions.

In severe hemodynamically significant coronary involvement, one of the options for restoring blood flow is coronary artery bypass grafting (CABG). After CABG, patients are prescribed antiplatelet therapy with acetylsalicylic acid (ASA) or clopidogrel to maintain patency of venous and arterial shunts. Despite the effectiveness of such antiplatelet treatment, in some patients there is a reduced sensitivity to both ASA and clopidogrel, which increases the risk of shunt thrombosis. Among the many reasons for platelet resistance to ASA, one of the main ones is the insufficient suppression of cyclooxygenase-1 (COX-1), which is involved in thromboxane A2 synthesis, which causes platelet aggregation. Resistance to clopidogrel is due to genetic polymorphisms of adenosine diphosphate (ADP) receptors and enzymes involved in medication metabolism, low adherence to therapy, and insufficient absorption.

Ultimately, the sensitivity of platelets is specified by their functional activity and activity of metabolic processes providing energy and plastic substrates during the life of these cells. With the harmonious work of all internal mechanisms, the receptors on platelet membrane are assembled, the active substances are synthesized and packed into granules, as well as the platelets are provided with energy resources. Here, an important role is played by reactive oxygen species (ROS), which can participate as signaling molecules, as well as activate platelets and their receptors [1]. ROS synthesis is associated with function of plasma membrane NADPH oxidase, myeloperoxidase, superoxide dismutase, and the inner mitochondrial membrane electron transport chain. In addition, the production of superoxide anion radical and hydroxyl radical is possible through receptor-mediated signaling pathways, as well as in the metabolism of arachidonic acid with the participation of COX-1 inside platelets, especially with collagen stimulation [2]. The resulting primary and secondary ROS can initiate further cascade of synthesis of other ROS. Since the ASA point of application is COX-1, it is likely that platelet susceptibility to it depends on the metabolism of arachidonic acid, which can be judged, in particular, by the production of ROS using chemiluminescence (CL) assay. To detect primary ROS, lucigenin, which does not penetrate into the cell, can be used, while for secondary ROS — luminol (passes through the cell membrane).

In the literature, there are studies revealing the variability in platelet sensitivity due to various reasons (cardiopulmonary bypass, renal dysfunction, selective non-steroidal anti-inflammatory drug therapy in the postoperative period, reduced bioavailability of enteric-coated ASA, inflammatory response) [3, 4].

Therefore, it seems interesting and extremely important to study the effect of CABG surgery on platelet sensitivity to ASA from the standpoint of their metabolic activity in relation to ROS production. At the same time, it is also necessary to assess the platelet production of ROS in patients resistant and sensitive to ASA, both with ASA monotherapy and with ASA+clopidogrel therapy after CABG.

Thus, the aim was to study the production of ROS by platelets in patients with coronary artery disease (CAD) before and after CABG, depending on their sensitivity to ASA as a part of ASA monotherapy and dual antiplatelet therapy (DAPT) (ASA+clopidogrel).

Material and methods

The study included 104 patients (79 men and 25 women) aged 35 to 76 years (mean age, 61±5,5 years) with Canadian Cardiovascular Society (CCS) class II-IV angina. Sixty-four patients took ASA monotherapy and 40 patients — DAPT (ASA+clopidogrel). All patients underwent CABG. Coronary artery atherosclerosis was confirmed by coronary angiography. The exclusion criteria were chronic kidney disease (glomerular filtration rate <60 ml/min/1,73 m^2), liver failure (exceeding the hepatic transaminase normal range by 3 or more times), gastric and/or duodenal ulcer, intolerance to ASA and clopidogrel. The characteristics of patients by clinical and laboratory characteristics are presented in Table 1.

Patients took the following drug groups before CABG: β-blockers — 91,9%, angiotensin-converting enzyme inhibitors — 90,8%, angiotensinogen II receptor blockers — 5%, calcium channel blockers — 16,9%, diuretics — 42,4%, aldosterone antagonists — 40,6%, statins — 100%. Before CABG, for 5 days, patients stopped receiving antiplatelet drugs, and from the first day after surgery they were prescribed with enteric-coated ASA 100 mg a day, while in the group of patients using DAPT, clopidogrel was prescribed by 2-3 days after CABG. Measurements throughout the study for each patient with CAD were carried out three times (before surgery, on day 1 immediately after surgery, and 8-10 days after surgery). This study design makes it possible to establish the effect of CABG and ASA intake on the studied parameters. The control group consisted of 36 healthy donors matched for sex and age.

All patients signed written informed consent. The study was carried out in accordance with the

Table 1

Patient characteristics

Characteristics	All patients	sASA	rASA	p
Sex, female/male, n (%)	25 (24%)/79 (76%)	14 (19,7%)/57 (80,3%)	11 (33,3%)/22 (66,7%)	0,131
Age (years), Me (C_{25}-C_{75})	63 (56-65)	62 (57-65)	62 (55-66)	0,319
Smoking (current status), %	39,7	42,5	29,6	0,227
Total cholesterol, mmol/l	4,28 (3,69-5,58)	4,50 (3,79-5,66)	3,93 (3,56-5,49)	0,132
Leukocytes, 10^9/l	7,57 (6,44-8,60)	7,60 (6,70-8,70)	7,10 (6,10-8,38)	0,774
Platelets, 10^9/l	228,0 (203,0-276,0)	222,0 (201,0-267,5)	229,0 (214,5-283,5)	1,0
Erythrocytes, 10^{12}/l	5,00 (4,7-5,3)	5,0 (4,7-5,3)	4,9 (4,7-5,2)	0,386
Hemoglobin, g/l	141,0 (133,0-152,3)	141,0 (133,5-154,5)	141,0 (132,0-147,0)	0,878
Creatinine, mmol/l	109,0 (97,8-119,0)	109,0 (98,5-118,5)	112,0 (97,0-119,0)	0,688
Class II angina, %	49,6	50,5	48,0	0,578
Class III angina, %	39,3	37,4	48,0	0,508
Diabetes, %	27,3	23,4	40,7	0,075
Prior myocardial infarction, %	62,8	62,8	62,9	0,986
Obesity, %	32,2	35,1	48,2	0,077

Note: data represent medians and interquartile range (Me (C_{25}-C_{75})). $p<0,05$ between the indices of patients with sASA and rASA (Mann-Whitney U-test). The χ^2 test was used to compare categorical variables, unless the expected frequencies in the contingency tables were less than 5, in which case Fisher's exact test was used.

Abbreviations: rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients.

Helsinki Declaration and Rules for Good Clinical Practice in Russia approved by Order of the Russian Ministry of Health dated June 19, 2003 № 266. The study protocol was approved by the Ethics Committee of V. F. Voino-Yasenetsky Krasnoyarsk State Medical University.

The study used blood stabilized with 3,2% sodium citrate in a 9:1 ratio. To determine the resistance of platelets to ASA, platelet-rich (blood centrifugation at 140 g for 10 min, at 24° C) and platelet-poor plasma (blood centrifugation at 1500 g for 15 min, temperature 24° C) were obtained. Determination of ASA resistance was carried out in 500 µl of platelet-rich plasma relative to platelet-poor plasma on a Chronolog 490 aggregometer (USA) with the addition of 5 µl of arachidonic acid (0,5 mM) (CHRONO-PAR, USA). If the measurement was carried out before ASA therapy initiation, then platelet aggregation with arachidonic acid was determined after their incubation with 10 µl ASA (3,36 mM, purity ⩾99,0%, A5376 Sigma Aldrich) *in vitro* for 3 min at 37° C to predict aspirin resistance. ASA resistance was determined at a level of platelet aggregation with arachidonic acid ⩾20% at least at one observation point: on antiplatelet therapy on days 8-10 after CABG or during incubation of the patient's plasma with ASA *in vitro* before the start of surgery and ASA treatment.

To determine the CL activity of platelets, platelet-rich plasma, after standing the blood in a thermostat for 30 min, was obtained by centrifuging stabilized blood at 140 g for 10 min at 24° C. The supernatant was collected and transferred into a plastic tube, adjusted with buffer № 1 (90 mM NaCl, 5 mM KCl, 36 mM sodium citrate, 10 mM EDTA, pH 762) to 2-fold dilution. The resulting mixture was centrifuged at 400 g for 15 min at 24° C. The pellet was resuspended in buffer № 1 (5 ml) and recentrifuged at 400 g for 1 min. The resulting supernatant was centrifuged again at 400 g for 15 min. The supernatant was carefully discarded; 10 ml of buffer № 2 (0,13 M NaCl, 0,02 M Tris-HCl buffer, 0,03 M EDTA, 0,015 M glucose, pH 7,4) was added to the sediment and centrifuged for 50 sec at 140 g. Evaluation of the number and purity of isolated platelets was carried out on a Sysmex XE-5000 hematology analyzer (Sysmex Inc., USA). The purity of isolated platelets was 98-100%. For the study, platelets were used in the amount of 2×10^7 cells per sample. The reaction mixture also included 50 µl of lucigenin or luminol at a concentration of 50 µg/ml, 50 µl of 0,1 M ADP (for assessing induced ROS synthesis) and 250 µl (for assessing spontaneous ROS synthesis) or 200 µl (for assessing ADP-induced ROS synthesis) of buffer (0,13 M NaCl, 0,02 M Tris-HCl buffer, 0,03 M EDTA, 0,015 M glucose, pH 7,4). Evaluation of spontaneous and ADP-induced CL was carried out for 90 min on a 36-channel biochemiluminescence analyzer BLM-3607 (OOO MedBioTech, Russia) [5].

Table 2

CL activity of platelets with lucigenin in patients with CAD before CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	219 (82-719)	813 (222-2841) p_1=0,027	611 (339-1908)	212 (70-560)
Imax, CU × 10^3	0,074 (0,06-0,086)	0,12 (0,09-0,50) p_1=0,006	0,061 (0,047-0,086) p_2=0,006	0,088 (0,07-0,096)
S, CU × sec × 10^6	0,225 (0,171-0,266)	0,30 (0,18-0,79)	0,136 (0,12-0,321)	0,227 (0,162-0,233)
Induced CL				
Tmax, sec	319 (73-1393)	1036 (445-3745) p_1=0,004	764 (509-1979)	636 (287-1201)
Imax, CU × 10^3	0,076 (0,062-0,082)	0,13 (0,08-0,43) p_1=0,013	0,071 (0,058-0,124)	0,1 (0,075-0,132)
S, CU × sec × 10^6	0,241 (0,161-0,295)	0,41 (0,25-1,11)	0,234 (0,18-0,344)	0,27 (0,202-0,312)
S_{ind}/S_{spont}	1,03 (0,96-1,24)	1,06 (0,89-1,28)	1,13 (1,01-1,21)	0,98 (0,93-1,65)

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

The following characteristics were determined: time to maximum plasma concentration (Tmax), maximum intensity (Imax), as well as the area under the CL curve (S). The enhancement of ADP-induced CL was assessed by the ratio of area under the induced CL curve of area under the spontaneous CL curve (S_{ind}/S_{spont}).

The sample description was made by calculating the median (Me) and interquartile range in the form of 25 and 75 percentiles (C_{25} and C_{75}). The statistical significance of differences between the independent samples was assessed by the nonparametric Mann-Whitney U test. Differences were considered significant at $p<0,05$. Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc., USA).

Results

Out of 104 patients with CAD, 71 (68,3%) were sensitive to ASA (sASA) throughout the study (46 patients, ASA monotherapy; 25 patients, ASA+clopidogrel). The remaining 33 (31,7%) patients with CAD showed resistance to ASA (rASA) at least at one follow-up point, which is consistent with other studies where resistance to ASA ranged from 5 to 50% [6-8]. Only 3 (9%) patients of all rASA patients with CAD were resistant during the entire observation period (1 patient, ASA monotherapy; 2 patents, ASA+clopidogrel therapy), and the sensitivity of remaining 30 rASA patients with CAD (17 patients, ASA monotherapy; 13 patents, ASA+clopidogrel therapy) changed in different follow-up periods, which is of interest for studying this phenomenon.

Study results showed that in sASA patients with CAD, many platelet CL parameters is significantly higher than in the control group. Before CABG, these are CL with lucigenin (Tmax and Imax in the spontaneous and ADP-induced test) and luminol (Imax in the spontaneous and ADP-induced test and S in the ADP-induced test). On the first day after CABG surgery, these are CL with lucigenin (Tmax and Imax in the spontaneous test) and luminol (Imax and S in the spontaneous test, Tmax and Imax in the ADP-induced test). On days 8-10 after CABG surgery, these are CL with lucigenin (Tmax and Imax in the spontaneous test, Imax and S in the ADP-induced test) and luminol (Imax and S in the spontaneous test, Tmax, Imax and S in the ADP-induced test) (Tables 2-7).

In the rASA patients with CAD (ASA monotherapy), compared with the control group, only the Tmax in the ADP-induced test with luminol before CABG was higher (Table 5). In the rASA group (ASA+clopidogrel), compared with the control group, the following CL indices were higher: Imax in the spontaneous test with lucigenin, Imax in the ADP-induced test and S in the spontaneous test with luminol (in the first days after CABG), as well as Imax in spontaneous and ADP-induced test with

Table 3

CL activity of platelets with lucigenin in patients with CAD on the first day after CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	219 (82-719)	494 (255-2043) p_1=0,008	408 (95-710)	212 (89-382)
Imax, CU × 10^3	0,074 (0,06-0,086)	0,1 (0,08-0,30) p_1=0,036	0,065 (0,05-0,077) p_2=0,006	0,097 (0,086-0,117) p_1=0,049
S, CU × sec × 10^6	0,225 (0,171-0,266)	0,35 (0,21-0,56)	0,182 (0,11-0,245)	0,36 (0,22-0,398)
Induced CL				
Tmax, sec	319 (73-1393)	458 (177-985)	509 (66-799)	282 (178-2519)
Imax, CU × 10^3	0,076 (0,062-0,082)	0,1 (0,08-0,45)	0,058 (0,054-0079) p_2=0,006	0,09 (0,072-0,097)
S, CU × sec × 10^6	0,241 (0,161-0,295)	0,32 (0,21-0,67)	0,176 (0,162-0,218)	0,314 (0,156-0,354)
S_{ind}/S_{spont}	1,03 (0,96-1,24)	1,03 (0,97-1,17)	0,92 (0,81-1,02)	0,89 (0,79-0,99) p_2=0,006

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

Table 4

CL activity of platelets with lucigenin in patients with CAD on days 8-10 after CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	219 (82-719)	611 (185-2198) p_1=0,015	212 (89-637)	124 (0-410) p_2=0,006
Imax, CU × 10^3	0,074 (0,06-0,086)	0,11 (0,09-0,34) p_1=0,002	0,064 (0,057-0,101)	0,085 (0,078-0,109)
S, CU × sec × 10^6	0,225 (0,171-0,266)	0,32 (0,20-0,85)	0,172 (0,158-0,199)	0,31 (0,244-0,329)
Induced CL				
Tmax, sec	319 (73-1393)	1625 (161-3099)	574 (171-819)	301 (53-1141)
Imax, CU × 10^3	0,076 (0,062-0,082)	0,12 (0,09-0,53) p_1=0,001	0,058 (0,051-0,097) p_2=0,006	0,085 (0,078-0,112)
S, CU × sec × 10^6	0,241 (0,161-0,295)	0,35 (0,28-1,47) p_1=0,037	0,13 (0,076-0,314) p_2=0,006	0,244 (0,145-0,315)
S_{ind}/S_{spont}	1,03 (0,96-1,24)	1,10 (0,88-1,95)	1,02 (0,44-1,36)	0,92 (0,51-1,1)

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

luminol (8-10 days after CABG surgery) (Tables 3, 6, 7).

In the rASA patients with CAD (ASA monotherapy) compared with the sASA ones, the S values in the ADP-induced test with luminol and Imax in the spontaneous test with lucigenin before CABG were significantly lower (Tables 2, 5). The same trend was observed on the first day after CABG for Imax in the spontaneous and ADP-induced test with lucigenin (Table 3), as well as on days 8-10

Table 5

CL activity of platelets with luminol in patients with CAD before CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	141 (0-680)	229,5 (40,2-1833,5)	255 (85-1655)	95 (71-319)
Imax, CU × 10^3	0,077 (0,057-0,085)	0,12 (0,08-0,55) p_1=0,01	0,06 (0,048-0,14)	0,081 (0,079-0,339)
S, CU × sec × 10^6	0,226 (0,12-0,285)	0,29 (0,2-0,95)	0,141 (0,09-0,26)	0,29 (0,21-0,35)
Induced CL				
Tmax, sec	95 (0-598)	454,5 (0-1800)	710 (234-2197) p_1=0,024	776 (61-1426)
Imax, CU × 10^3	0,07 (0,059-0,081)	0,113 (0,08-0,5) p_1=0,008	0,06 (0,056-0,098)	0,1 (0,072-0,434)
S, CU × sec × 10^6	0,222 (0,151-0,251)	0,3 (0,22-0,88) p_1=0,041	0,148 (0,132-0,277) p_2=0,006	0,313 (0,181-0,367) p_3=0,025
S_{ind}/S_{spont}	1 (0,88-1,1)	0,98 (0,75-1,13)	1,09 (1,01-1,19)	1,02 (0,67-1,11)

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD; p_3 — statistical significance of differences in comparison with rASA patients with CAD (ASA monotherapy).

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

Table 6

CL activity of platelets with luminol in patients with CAD on the first day after CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	141 (0-680)	141 (0-1107)	159 (90-2189)	141 (51-976)
Imax, CU × 10^3	0,077 (0,057-0,085)	0,15 (0,086-0,46) p_1=0,012	0,072 (0,049-0,088)	0,093 (0,084-0,185)
S, CU × sec × 10^6	0,226 (0,12-0,285)	0,33 (0,22-0,86) p_1=0,043	0,21 (0,084-0,328)	0,34 (0,299-0,444) p_1=0,015
Induced CL				
Tmax, sec	95 (0-598)	198 (0-1529) p_1=0,046	89 (0-1514)	71 (0-266)
Imax, CU × 10^3	0,07 (0,059-0,081)	0,130 (0,09-0,45) p_1=0,011	0,077 (0,053-0,103)	0,083 (0,078-0,293) p_1=0,024
S, CU × sec × 10^6	0,222 (0,151-0,251)	0,33 (0,17-1,05)	0,16 (0,011-0,234)	0,326 (0,155-0,36)
S_{ind}/S_{spont}	1 (0,88-1,1)	0,94 (0,83-1,1)	0,96 (0,84-1,17)	0,99 (0,81-1,19)

Note: p_1 — statistical significance of differences in comparison with control group.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

after CABG surgery for Imax in the ADP-induced test with luminol and lucigenin and S in the ADP-induced test with lucigenin (Tables 4, 7).

In rASA patients with CAD (ASA+clopidogrel) compared with sASA ones, the S_{ind}/S_{pont} values in lucigenin test on the first day after CABG surgery (Table 3), as well as Tmax in the ADP-induced test with luminol and Tmax in the spontaneous test with lucigenin on days 8-10 after surgery, CABG were significantly lower (Tables 4, 7).

Significant differences between rASA patients with CAD receiving ASA monotherapy and ASA+clo-

Table 7

CL activity of platelets with luminol in patients with coronary artery disease on days 8-10 after CABG (Me (C_{25}-C_{75}))

Parameters	Control (n=36)	sASA (n=71)	rASA (ASA monotherapy) (n=17)	rASA (ASA+clopidogrel) (n=13)
Spontaneous CL				
Tmax, sec	141 (0-680)	264 (0-1164)	382 (53-1134)	71 (0-660)
Imax, CU × 10^3	0,077 (0,057-0,085)	0,14 (0,09-1,253) p_1=0,001	0,06 (0,057-0,167)	0,134 (0,086-0,149) p_1=0,035
S, CU × sec × 10^6	0,226 (0,12-0,285)	0,4 (0,3-2,35) p_1<0,001	0,19 (0,171-0,416)	0,304 (0,172-0,358)
Induced CL				
Tmax, sec	95 (0-598)	492 (84,5-1876,5) p_1=0,01	355 (139-2211)	35,5 (0-279) p_2=0,006
Imax, CU × 10^3	0,07 (0,059-0,081)	0,18 (0,09-1,57) p_1<0,001	0,062 (0,052-0,12) p_2=0,006	0,117 (0,081-0,133) p_1=0,041
S, CU × sec × 10^6	0,222 (0,151-0,251)	0,41 (0,22-2,27) p_1=0,038	0,18 (0,126-0,409)	0,247 (0,131-0,33)
S_{ind}/S_{spont}	1 (0,88-1,1)	0,9 (0,62-1,5)	0,91 (0,57-1,03)	0,83 (0,6-1,14)

Note: p_1 — statistical significance of differences in comparison with control group; p_2 — statistical significance of differences in comparison with sASA patients with CAD.

Abbreviations: ASA — acetylsalicylic acid, CAD — coronary artery disease, CU — conventional units, rASA — acetylsalicylic acid-resistant patients, sASA — acetylsalicylic acid-sensitive patients, CL — chemiluminescence, Tmax — time to maximum chemiluminescence intensity, Imax — maximum chemiluminescence intensity, S — area under chemiluminescence curves.

pidogrel therapy were found only for S CL in the ADP-induced test with luminol before CABG (Table 5).

Discussion

The variability of platelet sensitivity to ASA depends on various factors, but many mechanisms of this phenomenon are still not fully understood. Platelets, as direct participants in not only hemostatic, but also immune and inflammatory processes, interact with a large number of cells and active molecules. At the same time, intercellular contacts of platelets with leukocytes are most pronounced in a chronic nonspecific inflammatory process, which, along with lipid disorders, underlies atherosclerosis. This can lead to a different response of platelets to ASA, depending on microenvironment. The increased basic and induced metabolic activity of platelets, manifested in high rates of production of primary and secondary ROS in sASA patients with CAD throughout the study, indicates a cell activeness and their potential upon additional stimulation. At the same time, both CABG surgery and antiplatelet therapy in some cases did not deplete internal resources of platelets, and the persisting high activity of platelets could initiate rethrombosis [9].

It is noteworthy that in the rASA patients with CAD (ASA monotherapy), the level and intensity of platelet ROS production does not differ from the control group. Only before CABG the time for reaching the maximum intensity of induced ROS production is increased, which indicates a slowdown in their production with additional stimulation. In the rASA patients with CAD (ASA+clopidogrel), a different picture is observed. After CABG surgery and while taking ASA and clopidogrel, platelets produce increased primary and secondary ROS relative to control values, which indicates the influence of these factors on platelet metabolism in this category of patients with CAD. Probably, residual platelet reactivity is due to stimulation of cells by cardiopulmonary bypass circuit, leukocytes, and production of new platelets 8-10 days after CABG [10]. Moreover, DATT does not reduce the production of ROS.

We have previously shown that in rASA patients with CAD (regardless of therapy), the level and intensity of ROS production by platelets is lower than in sASA ones [11]. The sensitivity is associated with increased metabolic activity of platelets for ROS synthesis, including for arachidonic acid (with COX-1 participation), which platelets can also receive from neutrophils during intercellular interaction [12]. In this study, this picture remains expressed in rASA patients with CAD (ASA monotherapy), while in the group of rASA patients with CAD (ASA+clopidogrel), only the time of ROS production was reduced and only by 8-10

days after CABG, which may be associated with the effect of clopidogrel on platelet receptors for ADP. In addition, platelets of sASA and rASA (ASA+clopidogrel) produce more secondary ROS upon stimulation before CABG surgery than r ASA patients with CAD (ASA monotherapy). These data indicate that the CL activity of platelets in rASA patients with CAD (ASA+clopidogrel) is at the level of sASA patients, i.e. using DAPT, it is possible to overcome the resistance to ASA.

Higher rates of ROS production in sASA patients with CAD and rASA (ASA+clopidogrel) may indicate that the NADPH oxidase activity in the platelets of these patients is higher than in rASA ones (ASA monotherapy). Thanks to DAPT, 2 pathways of platelet activation are blocked at once, but this can be compensated due to their increased contact with leukocytes both immediately after the operation and with taking antiplatelet drugs through the transfer of active substances through microvesicles, but not through the receptor-mediated leukocyte-platelet complex formation, since the number of such aggregates is significantly reduced along with the pro-inflammatory functions of platelets on clopidogrel therapy in patients with CAD [13]. Because of this, the amount of arachidonic acid, may increase, during the metabolism of which ROS is produced. And since in this group of patients with CAD, COX-1 resistance to ASA is observed, then high substrate levels contribute to increased formation of intermediate ROS products, and the main antiplatelet effect is provided not by ASA, but by clopidogrel. Purinergic receptors for ADP are also present on leukocyte cells. They are involved in many processes, including in leukocyte adhesion to endothelial cells during inflammation and atherosclerosis, therefore, they are also blocked by clopidogrel, which leads to disruption of intercellular bridges. In addition, leukocyte-platelet complexes are capable of producing ROS themselves, and the resulting nitric oxide can reduce platelet aggregation activity, thereby preventing recurrent thrombosis [14]. Therefore, in the group of rASA patients with CAD on DAPT, clinical outcomes may be more favorable due to clopidogrel presence. The addition of clopidogrel to ASA does not affect the very phenomenon of aspirin resistance, but it has an anti-inflammatory and additional antiplatelet function. And the probable disruption of intercellular contacts in rASA patients with CAD (ASA monotherapy) levels the antiplatelet effect of ASA due to the insufficient arachidonic acid amount in platelets, which causes a reduced sensitivity of such patients to ASA [15].

Conclusion

Thus, we obtained data indicating a difference in the metabolic activity of platelets in ROS production in CAD patients, depending on both the sensitivity to ASA and the options for antiplatelet therapy after CABG (ASA or ASA+clopidogrel). In sASA patients with CAD, ROS production is higher than in healthy individuals in the pre and postoperative period. In rASA patients with CAD treated with ASA monotherapy, platelets produce ROS levels lower than in sASA patients with CAD. However, CABG and the addition of clopidogrel to ASA therapy leads to increased ROS production in rASA patients in the postoperative period, which equates this group of patients with sASA patients in terms of ROS synthesis. These results may indicate a complete disruption of the intercellular interactions of platelets and leukocytes in rASA patients with CAD (ASA monotherapy) and partial (only at the receptor level) in patients with CAD (ASA+clopidogrel) with intact transport of substances using microvesicles, which is the result of blocking receptors by clopidogrel on both platelets and leukocytes.

Sensitivity to ASA may depend not only on COX-1 activity, but also on the presence and metabolic characteristics of enzyme substrate, on the activity of other cells that can interact with platelets, which, accordingly, depends on the environment (medication therapy, invasive interventions, comorbidities, etc.). Further study of these metabolic processes may lead to the discovery of additional mechanisms of aspirin resistance and ways to overcome this phenomenon.

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