# The influence of metabolic activity of platelets on sensitivity to Acetylsalicylic acid in patients with coronary heart disease

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**Background:** Acetylsalicylic acid (ASA) is used to reduce the risk of shunt occlusion after coronary artery bypass grafting (CABG). From 5% to 60% of CHD patients do not respond to ASA. This phenomenon was defined as ASA resistance. The functional activity of platelets is largely determined by the state of their metabolism.

**Methods:** The venous blood samples were acquired from 66 patients with CHD before CABG, on the first day after surgery, and on the 8–10th day after surgery. The aggregometry was carried out for all participants by an optical aggregometer with 1 mM of arachidonic acid (AA) and 5 mM Adenosinediphosphate (ADP). Resistance to ASA was determined at the level of platelet aggregation with AA over 20% on ASA therapy or over 20% after platelet incubation with ASA in vitro before CABG. Patients were divided into ASA sensitive (sASA) and ASA resistant (rASA) groups. The level of synthesis of primary and secondary reactive oxygen species (ROS) by platelets was determined using chemiluminescent analysis. We investigated the overall level of radical synthesis from the values of Imax and S (area under the chemiluminescence curve), which, respectively, characterizes the maximum synthesis per unit time and the total amount of radical. The kinetics of ROS synthesis was characterized by Tmax (time to reach the maximum for the chemiluminescent curve). The activity of NAD- and NADP-dependent dehydrogenases in platelets was determined by the bioluminescent method.

**Results:** It was found that the aggregation activity of platelets depended on the sensitivity of CHD patients to ASA and decreased during postoperative ASA therapy. The most pronounced differences in metabolic parameters of platelets in sASA and rASA patients were detected by Nox2 activity. Platelet aggregation activity was correlated with platelet Nox2 activity only in sASA patients and only before CABG. The level of AA-induced platelet aggregation with the addition of ASA in sASA patients before CABG was negatively correlated with the Imax of ADP-induced lucigenin-enhanced (r=–0.27, p=0.046) and S of spontaneous luminol-enhanced (r=–0.31, p=0.022) platelet chemiluminescence. Patients with rASA before CABG had positive correlations of AA-induced aggregation with S of spontaneous (r=0.63, p=0.029) as well as Imax (r=0.85, p<0.001) and S (r=0.87, p<0.001) of ADP-induced luminol-enhanced chemiluminescence of platelets. Therefore, the absence of correlations between platelet aggregation activity and Nox2 activity was determined by ASA resistance and postoperative ASA therapy. The synthesis of secondary ROS (Table) by platelets of CHD patients did not depend on the sensitivity of patients to ASA but increased during postoperative treatment with ASA. The activity of NAD(P)-dependent dehydrogenases in platelets did not differ in sASA and rASA patients with CHD.

**Conclusions:** Metabolic Activity of Platelets could influence on resistance to ASA in Patients with CHD.

Table. Chemiluminescent activity of platelet in patients with CHD before and after CABG.

| Parameters | Control 1 | Before CABG | | 1 Day After CABG | | 8–10 days after CABG | |
|---|---|---|---|---|---|---|---|
| | | sASA Patients 2 | rASA Patients 3 | sASA Patients 4 | rASA Patients 5 | sASA Patients 6 | rASA Patients 7 |
| Spontaneous lucigenin enhanced chemiluminescence | | | | | | | |
| Tmax, sec. | 213 (80–450) | 813 (88–2841)$^{*}$ | 185 (35–249)$^{\nabla}$ | 789 (283–2043)$^{*}$ | 66 (41–115)$^{\nabla}$ | 938 (565–1908)$^{*}$ | 88 (65–420)$^{\nabla}$ |
| Imax, r.u. | 80 (73–93) | 117 (78–566)$^{*}$ | 80 (72–92)$^{\nabla}$ | 105 (87–331)$^{*}$ | 94 (90–103)$^{*}$ | 173 (100–351)$^{*}$ | 106 (78–118)$^{*,\nabla,\$}$ |
| S, r.u. × sec. × $10^2$ | 2.38 (1.76–2.75) | 3.01 (1.79–7.74)$^{*}$ | 2.25 (1.95–3.13) | 4.05 (2.44–8.17)$^{*}$ | 2.38 (1.97–3.58)$^{\nabla}$ | 4.24 (2.75–6.58)$^{*}$ | 2.89 (2.22–3.66) |
| ADP induced lucigenin enhanced chemiluminescence | | | | | | | |
| Tmax, sec. | 96 (49–608) | 1036 (346–3743)$^{*}$ | 577 (342–1166) | 745 (355–1008)$^{*,\$}$ | 285 (217–341)$^{\nabla,\$}$ | 1266 (621–2198)$^{*}$ | 495 (263–1099) |
| Imax, r.u. | 80 (76–127) | 127 (84–498)$^{*}$ | 81 (73–109)$^{\nabla}$ | 360 (91–595)$^{*}$ | 98 (82–106)$^{\nabla}$ | 238 (119–455)$^{*}$ | 102 (81–129)$^{\nabla}$ |
| S, r.u. × sec. × $10^2$ | 2,75 (1.87–3.65) | 4.15 (2.51–10.89)$^{*}$ | 2.33 (2.06–3.32)$^{\nabla}$ | 4.86 (2.20–9.50)$^{*}$ | 3.05 (2.54–3.48) | 4.49 (3.35–7.31)$^{*}$ | 2.99 (2.49–3.80)$^{\nabla}$ |
| AI | 1.01 (0.86–1.87) | 1.12 (0.90–1.59) | 1.06 (0.92–1.40) | 1.11 (0.82–1.40) | 1.22 (0.91–1.37) | 1.22 (0.99–1.54) | 1.09 (1.02–1.17) |
| Spontaneous luminol enhanced chemiluminescence | | | | | | | |
| Tmax, sec. | 71 (0–464) | 230 (45–1748) | 71 (69–81) | 336 (71–998) | 852 (26–2394)$^{\$}$ | 269 (71–848)$^{*}$ | 54 (4–445)$^{\nabla}$ |
| Imax, r.u. | 80 (77–110) | 122 (80–611)$^{*}$ | 84 (80–381) | 205 (95–490)$^{*}$ | 137 (90–167)$^{*}$ | 561 (127–1116)$^{*,\$}$ | 165 (129–388)$^{*}$ |
| S, r.u. × sec. × $10^2$ | 2.62 (2.22–3.13) | 2.96 (2.10–8.99) | 3.01 (2.31–3.41) | 3.87 (2.71–9.70)$^{*}$ | 3.94 (3.20–4.75)$^{*}$ | 5.60 (3.96–12.35)$^{*,\$}$ | 3.63 (3.14–5.08)$^{*,\$}$ |
| ADP induced luminol enhanced chemiluminescence | | | | | | | |
| Tmax, sec. | 154 (0–471) | 455 (45–2197) | 68 (13–743)$^{*}$ | 634 (89–1567)$^{*}$ | 97 (69–241)$^{\nabla}$ | 631 (264–1483)$^{*}$ | 117 (28–530)$^{\nabla}$ |
| Imax, r.u. | 77 (71–100) | 113 (79–519)$^{*}$ | 90 (75–342) | 294 (96–785)$^{*}$ | 145 (88–188)$^{*}$ | 690 (156–1346)$^{*,\$}$ | 156 (120–398)$^{*,\$}$ |
| S, r.u. × sec. × $10^2$ | 2.35 (2.07–3.34) | 3.06 (2.24–8.35) | 3,27 (2.48–3.58) | 5.55 (2.37–11.51)$^{*}$ | 3.62 (3.20–5.25)$^{*}$ | 6.87 (3.87–20.11)$^{*,\$}$ | 4.74 (2.60–5.92) |
| AI | 0.99 (0.71–1.26) | 1.04 (0.73–1.45) | 1.10 (1.04–1.39) | 1.09 (0.84–1.32) | 1.00 (0.69–1.13) | 1.16 (0.85–1.37) | 1.06 (0.78–1.28) |

The data represent the medians and interquartile ranges (Me ($C_{25}$ – $C_{75}$)). $^{*}$: $p<0.05$ vs. control (Mann-Whitney *U*-test), $^{\nabla}$: $p<0.05$ between indicators of sASA and rASA patients in each period of the survey (Mann-Whitney *U*-test), $^{\$}$: $p<0.05$ vs. with indicators of the patients before CABG (Wilcoxon matched pairs test).