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Increased morning ADP-dependent platelet aggregation persists despite dual antiplatelet therapy in patients with first ST-segment elevation myocardial infarction: Preliminary report

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Abstract

Background: *Numerous trials have reported on the morning increase in the occurrence of myocardial infarction, stroke and sudden cardiac death. Similarly, enhanced morning platelet aggregation has been observed in healthy individuals and in subjects with coronary artery disease without adequate antiplatelet treatment. The purpose of the study was to assess circadian variation in platelet aggregation in patients with first ST-elevation myocardial infarction (STEMI) treated with primary percutaneous coronary interventions (pPCI) and dual antiplatelet therapy.*

Methods: *Fifteen consecutive patients (12 men and 3 women) were prospectively recruited into the study. Blood samples were collected at 6.00 a.m., 10.00 a.m., 2.00 p.m. and 7.00 p.m. on the third day of hospitalization. Aggregation in response to arachidonic acid and adenosine diphosphate (ADP) was assessed in the whole blood on a new generation impedance aggregometer.*

Results: *A morning increase of 75% in ADP-dependent platelet aggregation was noted in the study population ($p < 0.04$). In contrast, we failed to show any significant diurnal variation in arachidonic acid-mediated platelet aggregation. The magnitude of the morning surge in platelet aggregation after ADP stimulation did not correlate with its baseline level.*

Conclusions: *Increased morning ADP-dependent platelet aggregation persists despite dual antiplatelet therapy in patients with first STEMI undergoing pPCI. The clinical significance of this finding remains to be demonstrated.* (Cardiol J 2008; 15: 530–536)

Key words: platelet aggregation, circadian variation, antiplatelet therapy, acute myocardial infarction

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Introduction

Platelets play a pivotal role in the pathogenesis of acute coronary syndromes. Based on the impressive results of landmark trials, antiplatelet agents constitute the cornerstone of therapy in the acute phase as well as in secondary prevention in this setting [1, 2]. However, despite the widespread utilization of antiplatelet therapy, adverse cardiac events continue to occur in a substantial proportion of patients. In recent years significant inter-individual variability in response to antiplatelet drugs has been observed [3, 4]. Insufficient platelet inhibition was proposed to account for many of the ischemic complications. Furthermore, numerous reports linking both aspirin- and clopidogrel--resistance with unfavourable clinical outcomes supported this hypothesis [5–8]. On the other hand, hyperresponsiveness to aspirin and thienopyridines may pose a serious threat of bleeding. Due to the limited number of patients and events in these studies, it remains unclear whether platelet function testing should be routinely performed. In addition, optimal methods of measurement of platelet aggregation and cut-off values associated with high cardiovascular risk are yet to be determined. The relevance of these issues is growing, with the broad adoption of drug-eluting stents altering the vessel wall healing process that results in the necessity for prolonged dual antiplatelet therapy.

Most of the studies suggesting a close relation between enhanced platelet aggregation and unfavourable clinical outcome do not specify the exact timing of blood sampling [6–8]. This fact seems to be of a great importance, taking into account previous observations regarding increased morning platelet aggregation in healthy and hypertensive subjects, as well as in patients with coronary artery disease without adequate antiplatelet treatment, along with the phenomenon of morning excess of myocardial infarction, stroke and sudden cardiac death [9–15].

The purpose of the study was to assess circadian variation in platelet aggregation in patients with first ST-segment elevation myocardial infarction (STEMI) treated with primary percutaneous coronary interventions (pPCI) and dual antiplatelet therapy.

Methods

Study design and patients

Fifteen consecutive patients (12 men and 3 women) admitted to the Department of Cardiology and Internal Medicine of the Collegium Medicum in Bydgoszcz with a diagnosis of first STEMI and designated to undergo pPCI were prospectively recruited into the study. The enrolment criteria included: typical anginal pain at rest for at least 20 min, symptom onset less than 12 h before the admission to hospital and electrocardiographic features of currently evolving STEMI (elevation of ST segment ≥ 0.1 mV in at least two limb leads or ≥ 0.2 mV in at least two precordial leads). Trial exclusion criteria consisted of:

- age under 18 years or over 80 years;
- history of previous myocardial infarction;
- prior coronary revascularisation;
- cardiogenic shock at admission or initiation of the treatment with vasopressors before pPCI;
- bundle branch block;
- history of chronic heart failure in functional class III or IV of the New York Heart Association (NYHA), or hemodynamically significant valvular heart disease or idiopathic cardiomyopathy;
- history of cardiac pacing or indications for temporal cardiac pacing;
- persistent atrial fibrillation or other indication for oral anticoagulants;
- thrombocytopenia (< 100 000/mm^3) or history of congenital or acquired bleeding disorder;
- history of malignant neoplasm in the previous 5 years;
- recent trauma or major surgery (within the 2 months prior to enrolment);
- chronic obstructive pulmonary disease;
- any symptomatic concomitant infection;
- any concomitant immunosuppressive therapy including the use of steroids;
- chronic kidney disease defined as serum creatinine > 2 mg/dL or the need for renal replacement therapy.

All participants provided informed written consent. The clinical characteristics of the patient population are presented in Table 1. The study protocol was approved by the Local Ethics Committee.

Pharmacotherapy

At first contact with health care providers immediately after diagnosis of STEMI all patients were pretreated with an intravenous bolus of unfractionated heparin (70 IU/kg, but not more than 5000 IU) and oral loading doses of clopidogrel (600 mg) and aspirin (300 mg). At the catheterization laboratory a second dose of unfractionated heparin was intra-arterially administered in a weight-adjusted manner (up to 100 IU/kg) or under activated clotting time guidance (to the target range 200–250 s) if

Table 1. Clinical characteristics of the study population (n = 15).

Age [years]	56.0 (50.0–65.0)
Gender [male/female]	12/3
Infarct location: anterior/inferior/lateral wall	6/8/1
Time from symptom onset [h]	3.5 (2.5–8.0)
CK_{max} [U/L]	1589.0 (258.0–2506.0)
$CK\text{-}MB_{max}$ [U/L]	254.0 (50.0–361.0)
TnI_{max} [ng/ml]	36.7 (4.5–50.0)
LVEF [%]	47.5 (42.5–50.0)
Risk factors of CAD	
Body mass index [kg/m^2]	25.7 (24.9–31.9)
Arterial hypertension	6
Diabetes mellitus	5 including 3 newly diagnosed patients
Current smokers	9
History of smoking	4
Positive family history	4
Total cholesterol [mg/dl]	249.5 (201.0–268.0)
LDL cholesterol [mg/dl]	170.5 (121.0–203.0)
HDL cholesterol [mg/dl]	49.5 (39.0–58.0)
Triglycerides [mg/dl]	90.0 (61.0–120.0)

LVEF — left ventricular ejection fraction, CAD — coronary artery disease

Table 2. Angiographic and procedural characteristics of the study population (n = 15).

Coronary artery disease:	
Single-vessel	8
Multivessel	7
Localization of culprit lesion:	
Left anterior descending artery	4
Diagonal branch	1
Intermediate artery	1
Circumflex artery	1
Obtuse marginal artery	2
Right coronary artery	6
Baseline blood flow in the culprit vessel:	
TIMI 0	7
TIMI 1	2
TIMI 2	0
TIMI 3	6
Final blood flow in the culprit vessel:	
TIMI 3	15
Usage of abciximab	3
Direct stenting	12
Multivessel primary PCI	0
Number of implanted stents:	
1	13
2	2
Total length of implanted stents [mm]	18.0 (17.0–22.0)
Maximal stent or balloon diameter [mm]	3.5 (2.7–4.0)
Maximal inflation pressure [atm]	17.0 (16.0–20.0)
Outcome of primary PCI:	
Effective	15
Ineffective	0
Revascularization:	
Complete	10
Incomplete	5
Qualification for further treatment:	
Conservative	13
PCI	1
CABG	1

PCI — percutaneous coronary interventions; CABG — coronary artery bypass grafting

abciximab, a blocker of platelet glycoprotein IIb/IIIa, was intended. Abciximab was given at the discretion of the invasive cardiologist. Throughout the hospitalization period clopidogrel and acetylsalicylic acid were continued in single doses of 75 mg given at 8.00 a.m. Post-discharge antiplatelet therapy was planned in accordance with current European recommendations. Concomitant medications in all patients included ramipril and bisoprolol provided at 8.00 a.m. in doses adjusted for resting heart rate and blood pressure, as well as atorvastatin administered at 8.00 p.m.

Percutaneous coronary interventions

Coronary angiography and pPCI procedures were performed using the standard technique via the femoral artery with the aid of an Integris Allura device (Philips, the Netherlands). Non-ionic low-osmolar contrast media were applied. During angiography at least 5 left coronary artery and 3 right coronary artery projections were taken after a previous administration of 0.3 mg nitroglycerine into the coronary vessels, if arterial pressure was sufficient. Epicardial coronary flow was assessed according to the Thrombolysis in Myocardial Infarction (TIMI) scale. In all patients bare metal stents were implanted. The optimal direct effect of the intervention was assigned when no residual stenosis or a stenosis of less than 20% of the reference segment diameter was observed. Detailed characteristics of the procedures are displayed in Table 2.

Measurement of platelet aggregation

Blood samples were collected into hirudin-containing tubes at 6.00 a.m., 10.00 a.m., 2.00 p.m. and 7.00 p.m. The third day of hospitalization was chosen for blood sampling because at this time the patient with acute myocardial infarction is usually mobile, leaves the coronary care unit, and both aspirin and clopidogrel fully exert their antiplatelet properties. If a patient was admitted after 7.00 p.m., the next day was counted as the first day of hospital stay.

Platelet counts evaluated on the third day of hospitalization ranged from $125 \times 10^3/\mu L$ to $317 \times \times 10^3/\mu L$, with a median value of $220 \times 10^3/\mu L$,

whereas mean platelet volume was 10.8 fL (range 10.2–13.3 fL). Aggregation in the whole blood was assessed within 2 h of the venipuncture on a new generation impedance analyzer with multiple electrode aggregometry according to the manufacturer's instructions [16]. This method is capable of detecting the effect of both clopidogrel and aspirin treatment, and its results prior to, and after, antiplatelet treatment correlate well with light transmission aggregometry [17]. The whole procedure of platelet aggregation measurement with a Multiplate® (Dynabyte, Munich, Germany) device was performed in approximately 10 min. Whole blood, which was utilized in our study, is the physiological environment in which platelet function takes place in vivo. Moreover, the use of whole blood for *in-vitro* testing eliminates the need for the time-consuming centrifugation steps required to obtain platelet-rich plasma necessary for light transmission aggregometry. Therefore, it must be stressed that impedance aggregometry and light transmission aggregometry measure different aspects of platelet function. Impedance aggregometry results reflect interactions between platelets, red and white cells, while light transmission aggregometry does not [18]. Dyszkiewicz-Korpanty et al. [19] even suggests that whole blood aggregation appears to be more sensitive in detecting clopidogrel effects compared with the platelet-rich plasma methods. The principle of impedance aggregometry is based on the fact that platelets get sticky upon activation, and therefore have a tendency to adhere and aggregate on metal sensor wires in the test cell. One Multiplate® test cell incorporates 2 independent sensor units, each consisting of 2 silver-coated, highly conductive wires. When activated platelets adhere to the sensor wires the electrical resistance between the wires rises, which is continuously registered. The instrument detects the impedance change of each sensor separately and transforms it to arbitrary aggregation units (AU) that are plotted against time. The area under the aggregation curve (AUC) is the estimator of platelet aggregation that was evaluated in our study. It is affected by the total height of the aggregation curve as well as by its slope and is best suited to express the overall platelet activity. The aggregation quantified as the area under the curve is displayed in arbitrary units (10 AU × min = 1 U). In previous studies AUC has been highlighted as the parameter with the highest diagnostic power [16, 20]. To assess platelet response to aspirin and clopidogrel, we applied ASPItest and ADPtest (Dynabyte, Munich, Germany), respectively. The former uses arachidonic acid that serves as the substrate of the cyclooxygenase for synthesis of the potent platelet agonist, thromboxane A2, while in the latter, adenosine diphosphate (ADP) stimulates platelet activation by the ADP receptors that are blocked by clopidogrel. Using this fast and standardized method, comprehensive information on platelet function and antiplatelet therapy can be obtained. Reported intra-assay coefficients of variation (CV) were 11.5% for ASPItest and 14.1% for ADPtest, while intra-individual CVs were 11.4% for ASPItest and 13.7% for ADPtest, respectively [18]. The producer recommends 30 U and 50 U as the cut-off values associated with platelet hyperaggregability in patients on aspirin and clopidogrel therapy, respectively.

Statistical analysis

The use of the Shapiro-Wilk test demonstrated that the investigated variables were not normally distributed. Therefore, continuous results were reported as median values and interquartile ranges. Multiple comparisons were analysed with the ANOVA Friedman test, whereas the Wilcoxon matched--paired rank sum test was used for comparisons between 2 sampling points. Correlations were tested with the Spearman rank correlation test. A value of $p < 0.05$ was considered statistically significant; $0.05 \geq p < 0.1$ was regarded as a trend towards statistical significance, while $p \geq 0.1$ was marked as ns. All computations were carried out with Statistica, version 8.0 (StatSoft, Tulsa, USA).

Results

A substantial circadian variation in ADP-dependent platelet aggregation in subjects with STEMI treated in line with contemporary clinical practise was observed. Median peak values at 10.00 a.m. were 75% higher than those measured in the early morning ($p < 0.04$). A subsequent drop in platelet aggregation in response to ADP as an agonist was noted at 2.00 p.m. (Fig. 1). Levels of platelet aggregation assessed at 6.00 a.m., 2.00 p.m. and 7.00 p.m. in the ADP test were comparable. The ANOVA Friedman test indicated a borderline heterogeneity among circadian levels of ADP-dependent platelet aggregation ($p = 0.073$). In contrast, we did not find any statistically significant diurnal variation in arachidonic acid-dependent platelet aggregation, despite a visual tendency towards increased morning aggregability (Fig. 2). However, the magnitude of augmentation in this case was considerably lower than that seen for ADP-dependent platelet aggregation (44% *vs*. 75%). Baseline results of both

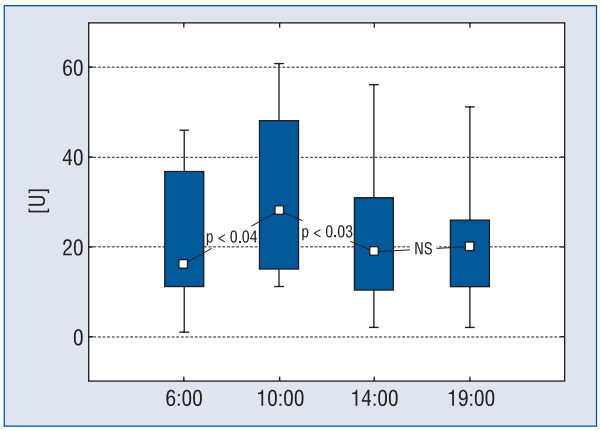

Figure 1. Circadian variation in adenosine diphosphate -dependent platelet aggregation assessed on the 3rd day of hospitalization in patients with ST-segment elevation myocardial infarction treated with dual antiplatelet therapy.

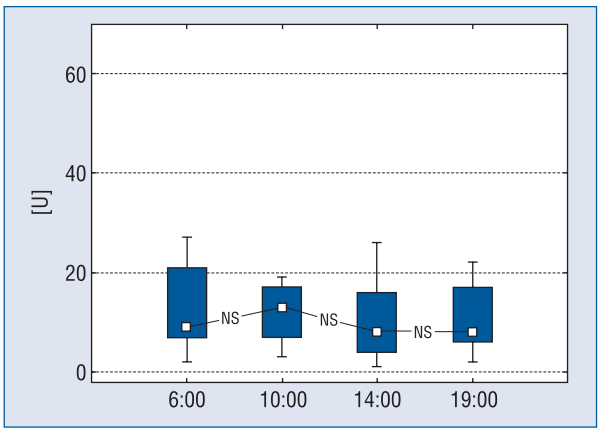

Figure 2. Circadian variation in arachidonic acid-dependent platelet aggregation assessed on the 3rd day of hospitalization in patients with ST-segment elevation myocardial infarction treated with dual antiplatelet therapy.

ADP and ASPI tests did not correlate with their highest levels. Aggregation measurements at each time point were unrelated to the platelet count as well as mean platelet volume. The vast majority of the ADP test results and all ASPI test results in our patients were below the cut-off values that, according to the producer, may be associated with aspirin and clopidogrel resistance, respectively.

Discussion

Numerous trials have reported on the morning increase in the occurrence of myocardial infarction, stroke and sudden cardiac death. This well-known phenomenon seems to be attributed to numerous pathomechanisms such as the morning surge in platelet aggregability accompanied by a trough in the fibrinolysis system, an increased morning thrombin formation coexisting with a morning peak in blood pressure triggered by sympathetic overactivity and cortisol hypersecretion [21]. In particular, the extensive morning release of catecholamines promotes thrombus formation and enhances myocardial vulnerability. Previous studies indicate that assuming an upright posture and initiation of daily activities occupy a crucial role in morning platelet hyperaggregability in healthy subjects [9, 22]. A particularly pronounced platelet hyperresponsiveness to physical exercise appears in habitually inactive subjects [23]. Andrews et al. [24], in a sophisticated flow cytometric study, proved that the morning increase in platelet aggregation does not occur with expression of activation-dependent platelet surface receptors. In another experimental work these researchers successfully attenuated the morning orthostatic increase in platelet aggregation by an $alpha_2$-adrenergic blockade with yohimbine [25]. Similarly, guanabenz, a centrally acting $alpha_2$-agonist exerting antihypertensive properties, suppressed morning elevations in aggregation of human platelets [26]. However, Willich et al. [27] showed in a well-designed study that $alpha_2$-adrenergic receptor density and agonist binding affinity assessed simultaneously did not change after arising. They also suggested that the increase in platelet aggregability is due to factors extrinsic to the platelets or to an intra-platelet mechanism distal to the receptor level.

According to our knowledge, we demonstrated for the first time that increased morning ADP-dependent platelet aggregation persists despite dual antiplatelet therapy in patients with first ST-segment elevation myocardial infarction. On the other hand, we failed to show any statistically significant circadian variation in platelet aggregation in response to arachidonic acid in this group, despite a visual tendency towards differentiated diurnal platelet response to aspirin, as illustrated in Figure 1. Moreover, the magnitude of the morning surge in platelet aggregation after ADP stimulation was not associated with its baseline level.

Several facts should be taken into account when interpreting our findings. A large body of evidence has emerged which highlights the limited potential of aspirin in terms of antiplatelet properties. Larsson et al. [28] indicated that norepinephrine-induced platelet activation in vivo is only partly counteracted by aspirin in normal subjects.

Similarly, Rinder et al. [29] failed to inhibit by aspirin the adenosine diphosphate-induced platelet alpha-granule release. Furthermore, in another experiment fibrinogen binding to unstimulated platelets or to platelets stimulated with ADP or thrombin was unaffected by aspirin in healthy subjects [30]. Additionally, aspirin treatment in the normal population did not attenuate platelet or leukocyte activation as monitored by whole blood flow cytometry [31]. Finally, in a Swedish study aspirin only modestly suppressed the prothrombotic effects triggered by vigorous exercise [32]. Contrary to these data, enteric-coated aspirin markedly reduced baseline platelet thromboxane A2 production in healthy males and eliminated its increase after the subjects got up [33]. It also abolished biphasic aggregation in response to epinephrine and ADP. The lack of morning increase in thromboxane A2 synthesis observed by McCall et al. [33] corresponds with our results, indicating suppression of the circadian variation in thromboxane A2-dependent platelet aggregation. On the other hand, the enhanced morning response to ADP that persisted in our trial despite the addition of clopidogrel contrasts with the findings of American researchers. However, it must be stressed that both studies examined different populations: healthy subjects vs survivors of myocardial infarction reflecting the opposite ends of the thrombotic milieu.

The limitations of clopidogrel therapy are currently widely discussed. The major drawbacks of clopidogrel include delayed onset of action, modest antiplatelet effect and substantial interpatient variability. Perneby et al. [34] reported that clopidogrel treatment in healthy volunteers attenuated platelet activity in vivo at rest, but exercise counteracted the platelet stabilizing effects of clopidogrel. The authors also extended their observations to patients with stable coronary artery disease. In this group, dual antiplatelet treatment did not reduce ECG signs of either exercise-induced or ambulatory myocardial ischemia [35]. Moreover, Eikelboom et al. [36] suggest that the clinical benefits of clopidogrel are not associated with a parallel reduction in markers of coagulation activation such as P-selectin, prothrombin fragment F1.2, D-dimer or von Willebrand factor.

Due to the limited number of patients in our trial, we plan to validate our results in a larger cohort. If our findings confirm, further studies to clarify their clinical significance are warranted. We are of the opinion that the periodicity in platelet aggregation present on antiplatelet therapy should be adjusted for the discriminative ability of various methods applied in the analysis of platelet function. Pepine [21] suggests that attention to the vulnerable morning period is merited in the timing and choice of medication, both to prevent or reduce ischemia and to modify potential disease-triggering mechanisms. We speculate that suppression of the morning increase in platelet aggregability by novel potent antiplatelet agents may further improve the long-term prognosis in survivors of myocardial infarction. The results of the Physicians' Health Study assigning 22 071 US male physicians to aspirin (325 mg daily) or placebo support this hypothesis [37]. Ridker et al. [37] reported that aspirin use when compared to placebo was associated with a 59.3% reduction in the incidence of infarction during the morning waking hours, compared with a 34.1% reduction for the remaining hours of the day. Nevertheless, we feel obligated to emphasize that, despite a temporal association between increased platelet aggregability in the morning and an increased frequency of myocardial infarction and stroke and sudden cardiac death, a cause-effect relation has been suggested but never proven [9]. In our study we applied impedance aggregometry. As a consequence of the fact that the Multiplate® is a newly invented device, the data assessing its diagnostic power are still sparse. Nevertheless, in a study by Mengistu et al. [38] impedance aggregometry with the Multiplate® device, but not thromboelastography, successfully predicted the postoperative requirements for blood transfusion in patients undergoing cardiac surgery. Similarly, a recent trial including 50 consecutive patients scheduled for neuro-interventional stent placement procedures demonstrated an unfavourable clinical outcome in clopidogrel nonresponders [39].

To conclude, increased morning ADP-dependent platelet aggregation persists despite dual antiplatelet therapy in patients with first ST-elevation myocardial infarction. The clinical significance of this finding remains to be demonstrated.

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