Collagen-induced platelet reactivity assessed by multiple electrode aggregometry in patients on dual antiplatelet therapy or aspirin monotherapy

Reaktywność płytek krwi indukowana kolagenem oceniana metodą agregometrii wieloelektrodowej (MEA) u pacjentów stosujących podwójną terapię przeciwplażkową lub monoterapię aspiryną

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Abstract

Introduction: Multiple electrode aggregometry (MEA) is used to assess platelet function and reactivity. This method allows for monitoring of antiplatelet therapy in patients and is important in the preoperative and perioperative periods, especially in patients after coronary artery bypass grafting (CABG).

Aim: The aim of this study was to evaluate whether collagen-induced aggregation is more diagnostic than standard agonists (arachidonic acid or ADP) in patients receiving dual antiplatelet therapy (DAPT) or aspirin monotherapy (AM) after CABG.

Materials and methods: The study included 155 patients with multi-vessel coronary artery disease and after CABG who were on antiplatelet therapy (aspirin 75 mg/day and clopidogrel 75 mg/day or aspirin 150 mg/day). Platelet aggregation in the blood of CABG patients, in response to arachidonic acid (0.5 mmol/L), collagen (3.2 μg/mL) and ADP (6.4 μmol/L) was assessed using a Multiplate® analyser.

Results: Platelet aggregation induced by collagen, ADP, and arachidonic acid was statistically significantly higher in AM patients compared to DAPT patients (p<0.03, p <0.0001 and p<0.0001, respectively). Furthermore, collagen-dependent platelet aggregation was only partly inhibited in both groups.

Conclusions: The use of traditional platelet agonists, such as ADP or arachidonic acid, is not sufficient to monitor antiplatelet therapy. Studies should be supplemented with additional platelet activation factors, such as collagen, to identify other receptors that may be important for antiplatelet therapy in cardiac patients.

Keywords: aggregation, aspirin monotherapy, collagen, dual antiplatelet therapy, MEA, platelet function test, platelet reactivity
Streszczenie

Wprowadzenie: Agregometria wieloelektrodowa (MEA) służy do oceny funkcji i reaktywności płytek krwi. Metoda ta pozwala na monitorowanie terapii przeciwłupkowej u pacjentów i jest ważna w okresie przedoperacyjnym i okołooperacyjnym, szczególnie u pacjentów po pomostowaniu aortalno-wieńcowym (CABG).

Cel: Celem pracy była ocena agregacji płytek krwi indukowanej kolagenem w porównaniu ze standardowymi agonistami (kwas arachidonowy, ADP) u pacjentów po CABG, stosujących podwójną terapię przeciwłupkową (DAPT) lub monoterapię aspiryną (AM).

Material i metody: Do badania włączone 155 pacjentów z wielonaczyniową chorobą wieńcową po CABG, stosujących terapię przeciwłupkową (aspiryna 75 mg/dobę i klopidogrel 75 mg/dobę lub aspiryna 150 mg/dobę). Agregację płytek krwi w krwi pełnej przeprowadzono przy użyciu analizatora Multiplate®. Do oceny reaktywności płytek krwi używano następujących agonistów: kwas arachidonowego, kolagenu i ADP.

 Wyniki: Agregacja płytek krwi pod wpływem kolagenu, ADP i kwasu arachidonowego była istotnie statystycznie większa u pacjentów AM w porównaniu z pacjentami DAPT (odpowiednio: p<0,03, p<0,0001, p<0,0001). Ponadto zależna od kolagenu reaktywność płytek krwi nie została wystarczająco zahamowana zarówno w grupie AM, jak i DAPT.

Wnioski: Zastosowanie wyłącznie tradycyjnych agonistów, takich jak ADP czy kwas arachidonowy, nie jest wystarczające, ponieważ w naszym badaniu wykazałyśmy niepełne zahamowanie agregacji płytek krwi indukowanej kolagenem. Istnieje zatem potrzeba dalszych badań w celu identyfikacji leków blokujących receptory kolagenowe, takie jak glikoprotein VI (GPVI).

Keywords: agregacja, kolagen, MEA, monoterapia aspiryną, podwójna terapia przeciwłupkowa, reaktywność płytek krwi, test funkcji płytek krwi

INTRODUCTION

Platelet function tests are used for various purposes, such as investigating patients who are prone to bleeding or taking antiplatelet drugs [1, 2]. Among such tests, light transmission aggregometry (LTA) and impedance aggregometry are widely applied techniques. In contrast to LTA, impedance aggregometry requires whole blood. Impedance aggregation has several advantages: no pre-analytical phase involving the preparation of platelet-rich plasma is required, only a small volume of blood is needed and the conditions are more physiological. Although the sensitivity of impedance aggregometry may not be very high in the detection of mild platelet dysfunction, some studies have shown that impedance aggregometry results are very similar or even more precise than LTA results for the detection of severe platelet dysfunction [1-3]. Impedance platelet aggregation can be replaced by another platelet function test developed to monitor platelet response to antiplatelet therapy (VerifyNow, PFA-100, etc.) [4-6]. However, it is still unclear whether all these methods are truly useful for monitoring antiplatelet therapy, as they are not equally effective in measuring the effects of antiplatelet drugs [1, 7].

Impedance-based multiple electrode aggregometry (MEA) is used in four cases to assess platelet function in patients after coronary artery bypass grafting (CABG). The first, and most common case, is evaluating the ability of platelet aggregation to reduce the time to release for CABG in patients with acute coronary syndrome who are on dual antiplatelet therapy [8, 9]. The second case is the testing of platelet reactivity to predict the risk of postoperative bleeding [10-12]. Another one concerns the early postoperative period of CABG [13]. The least common use of MEA, however, is for long-term adjustment of antiplatelet therapy after CABG in the late postoperative period [14]. The diagnostic and prognostic value of multi-plate impedance aggregation for monitoring (tailoring) antiplatelet therapy is still controversial [15]. Platelet function analysis using this method has been demonstrated by the TROPICAL-ACS trial to be beneficial for monitoring antiplatelet drug therapy [16].

For obvious reasons, ADP- and arachidonic acid (AA)-induced platelet aggregation assays are performed on the blood of patients treated with antiplatelet therapy [17]. Manufacturers of laboratory reagents have discontinued the sale of collagen in kits for the assessment of platelet reactivity, but an open question remains regarding the use of this agonist in the diagnosis of platelet disorders and, in the longer term, the evaluation of the effects of new generations of antiplatelet drugs [3, 18], including platelet glycoprotein VI (GPVI) antagonists [19].

The aim of this study was to compare collagen-induced platelet aggregation with platelet aggregatory response to standard platelet agonists (i.e. ADP and AA) in patients after CABG surgery treated with dual antiplatelet therapy (DAPT) or aspirin monotherapy (AM).
MATERIAL AND METHODS

Study group

In this cohort study, 155 patients (aged 61.4 ±8.0 years) with multi-vessel coronary artery disease who underwent elective or urgent CABG were included. Patients with type 2 diabetes accounted for 19.4% of the study group. Detailed characteristics of the enrolled patients are presented in Table I. All patients underwent standard surgical revascularization using cardiopulmonary bypass (CPB).

Table I. Baseline characteristics of the study cohort.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>CABG PATIENTS (N = 155)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>61.4 ±8.0</td>
</tr>
<tr>
<td>Male sex</td>
<td>121 (78.1)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>30 (19.4)</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>143 (92.3)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>131 (84.5)</td>
</tr>
<tr>
<td>Hb, g/dL</td>
<td>14.6 ±1.2</td>
</tr>
<tr>
<td>PLT, ×10^9/L</td>
<td>214.5 ±54.4</td>
</tr>
<tr>
<td>MPV</td>
<td>9.2 ±0.9</td>
</tr>
<tr>
<td>WBC, ×10^9/L</td>
<td>7.3 ±1.9</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.4 ±2.4</td>
</tr>
<tr>
<td>Aspirin</td>
<td>155 (100.0)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>66 (42.6)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>146 (94.2)</td>
</tr>
<tr>
<td>Statins</td>
<td>155 (100.0)</td>
</tr>
</tbody>
</table>

Two different antiplatelet treatment regimens were evaluated, depending on the patient’s medical history: aspirin monotherapy (150 mg acetylsalicylic acid [ASA] per day) and aspirin (75 mg ASA per day) in combination with clopidogrel (75 mg per day). The mean follow-up time ranged from 6 to 12 months after CABG. Bleeding complications related to antiplatelet therapy were reported during the study period.

The exclusion criteria were as follows: a platelet count of <100×10^9/L or >400×10^9/L, a glomerular filtration rate of <60 mL/min/1.73 m^2, and an age of >75 years, treatment with direct-acting oral anticoagulants (DOACs) or Vitamin K antagonists and taking any anti-inflammatory drugs within two weeks of blood collection. The on-treatment platelet reactivity of each patient was assessed from 6 to 12 months after CABG intervention.

The study was performed under the guidelines of the Helsinki Declaration for human research and approved by the Bioethics Committee of the Medical University of Łódź (No. RNN/21/04/KE). All participants confirmed their voluntary and conscious participation in the study.

Blood sampling

For platelet aggregation assays, blood was drawn by peripheral vein puncture into plastic tubes (S-Monovette; Sarstedt, Nümbrecht, Germany) containing 250 µg/mL of hirudin (Refludan®; Schering AG, Germany), which resulted in a final hirudin concentration of 25 µg/mL in the blood sample. Whole blood was stored at room temperature. Blood samples for whole blood count were drawn into tubes containing the anticoagulant K2EDTA. All samples were analysed within 2 hours of blood collection.

Multiple electrode aggregometry (MEA)

A semiautomatic, 5-channel impedance aggregometer (Multiplate® Analyser; Roche Diagnostics GmbH, Mannheim, Germany) was used to assess platelet aggregation in whole blood. Whole blood, drawn into a tube with an anticoagulant (hirudin), was diluted and then mixed in test cuvettes for 3 min.

The platelet aggregation induced by the three following agonists was then measured: arachidonic acid (ASPItest; final concentration: 0.5 mmol/L), ADP (ADPtest; final concentration: 6.4 µmol/L) and collagen (COLtest; final concentration: 3.2 µg/mL). The increase in impedance caused by the adhesion of the platelets to the metal electrodes was observed for 6 min. Results are presented as area under the curve over time, measured in arbitrary units (U).

Reference ranges for MEA platelet aggregation

Platelet aggregation induced by three agonists (collagen, ADP and arachidonic acid) was measured in 100 healthy individuals (50 women and 50 men; mean age 39 ±11 years) whose mean platelet counts ranged from 211 to 273 ×10^9/L. For 7 days prior to the start of the experiment, the participants did not take any medications that could affect platelet function, nor did they have any signs of inflammation [20].

The reference ranges for the magnitude of platelet aggregation induced by the aforementioned agonists (Tab. II) that were determined in the healthy subjects was used to interpret and compare the data obtained for the AM and DAPT patient groups. When platelet reactivity reached a value within the reference range, the result was classified as insufficiently inhibited platelet reactivity.

Table II. Platelet aggregation induced by collagen, ADP and arachidonic acid in healthy subjects (n = 100).

<table>
<thead>
<tr>
<th>PLATELET FUNCTION TEST</th>
<th>MEAN</th>
<th>MEDIAN</th>
<th>P5-P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLtest</td>
<td>78</td>
<td>74</td>
<td>52-117</td>
</tr>
<tr>
<td>ADPtest</td>
<td>73</td>
<td>72</td>
<td>50-104</td>
</tr>
<tr>
<td>ASPItest</td>
<td>78</td>
<td>78</td>
<td>41-103</td>
</tr>
</tbody>
</table>

Abbreviations: P5 – 5° percentile, P95 – 95° percentile
Data reflect the pooled results expressed as area under the curve (AUC).
Statistical analysis

Values for mean and standard deviation are given for normally distributed variables. Medians and interquartile ranges (Me, IQR: from 25% quartile to 75% quartile) are given for non-normally distributed parameters, as evaluated by the Shapiro-Wilk W test. Simple paired comparisons were performed using an unpaired Student’s t test, while non-normally distributed variables were analysed using the Mann-Whitney U test. For categorical data, Pearson’s χ² test with Yates correction (if necessary) was performed. Differences between the variables were considered to be statistically significant when the P-value was < 0.05. The statistical analysis was performed in Statistica 13.1 software (Statsoft, Cracow, Poland) and with StatsDirect statistical software, version 2.7.8 (StatsDirect Ltd, Altrincham, UK).

RESULTS

Characteristics of patients in the DAPT and monotherapy aspirin groups

Table III shows the most important parameters that describe patients treated with aspirin only (AM group) or aspirin in combination with clopidogrel (DAPT group).

Table III. Characteristics of CABG patients (n = 155) allocated to subgroups with aspirin monotherapy and dual antiplatelet therapy.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>ASPIRIN MONOTHERAPY (N = 89)</th>
<th>DUAL ANTIPLATELET THERAPY (N = 66)</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>60.9 ±7.9</td>
<td>62.1 ±8.1</td>
<td>ns</td>
</tr>
<tr>
<td>Male sex</td>
<td>72 (80.9)</td>
<td>60 (90.9)</td>
<td>ns</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.1 ±4.3</td>
<td>28.2 ±4.6</td>
<td>ns</td>
</tr>
<tr>
<td>Diabetes</td>
<td>19 (21.3)</td>
<td>11 (16.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>79 (88.8)</td>
<td>64 (97.0)</td>
<td>ns</td>
</tr>
<tr>
<td>Hypertension</td>
<td>71 (79.8)</td>
<td>60 (90.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Perioperative MI</td>
<td>5 (5.6)</td>
<td>7 (10.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>EuroSCORE points</td>
<td>6.1 ±2.2</td>
<td>6.7 ±1.9</td>
<td>ns</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.5 ±2.4</td>
<td>2.2 ±2.4</td>
<td>ns</td>
</tr>
<tr>
<td>Hb, g/dL</td>
<td>14.8 ±1.3</td>
<td>14.3 ±1.2</td>
<td>ns</td>
</tr>
<tr>
<td>WBC, ×10^9/L</td>
<td>6.8 ±1.8</td>
<td>6.8 ±1.9</td>
<td>ns</td>
</tr>
<tr>
<td>PLT ×10^9/L</td>
<td>191.1 ±49.3</td>
<td>213.8 ±61.4</td>
<td>ns</td>
</tr>
<tr>
<td>MPV, fL</td>
<td>9.0 ±0.9</td>
<td>9.4 ±1.0</td>
<td>ns</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean ± standard deviation or as medians (interquartile range). Categorical data are presented as absolute numbers (percentages). The Mann-Whitney U test (continuous data) or Fisher’s exact test (categorical data) was used to assess the significance of differences. Abbreviations: BMI body mass index, CRP – C-reactive protein, Hb – hemoglobin, MI – myocardial infarction, MPV – mean platelet volume, WBC – white blood cells

DISCUSSION

The use of perioperative tests to analyse platelet function is not yet recommended [1], so we did not focus on monitoring antiplatelet therapy in this study. Our main objective was to evaluate platelet reactivity in response to an agonist such as collagen.

Collagen is not used to assess the inhibition of platelet reactivity induced by traditional antiplatelet drugs (ASA and P2Y12 inhibitors/antagonists), so this laboratory product is no longer being sold, a situation which has caused some consternation among platelet specialists and has started a discussion on the need for commercial access to this reagent [18].

The results of our study suggest that there is a gap in the management of patients requiring antiplatelet treatment. Collagen-dependent platelet reactivity was not sufficiently inhibited in either the AM (n = 89) or DAPT (n = 66) groups. In our study we observed a high efficacy of DAPT therapy, but the mean collagen-induced platelet aggregation in the study group was still in the normal range (>5th percentile). The mean collagen-induced platelet aggregation in the AM group was significantly higher than that of the DAPT group. Incomplete inhibition of platelet function by antiplatelet drugs (generally aspirin and clopidogrel) is frequently observed in patients after CABG [21-23].

The Multiplate® device has been shown to be the only reliable and acceptable method for measuring arachidonic acid-induced platelet function in healthy volunteers and donors on daily antiplatelet therapy [24]. The results we have obtained from the experiments on platelet reactivity in healthy individuals are presented in separate publications [20, 25]; these data came from a project completed in 2018 [25]. Unfortunately, our platelet aggregation experiments performed on the blood of healthy subjects using a panel of platelet agonists were discontinued when collagen was withdrawn from the market. The previously established reference ranges for MEA platelet aggregation using ADP, AA and collagen are presented in Table III. It can be seen that the results differ from the previously published data [26], including the reference ranges suggested by the manufacturer,
established reference ranges from a large number of healthy volunteers for platelet aggregation in response to common agonists [26]. According to this data, the reference value for collagen-induced platelet aggregation measured in hirudin-anticoagulated blood was 48-112 U [26].

Overall, our results suggest that collagen-induced platelet aggregation is insufficiently inhibited in both the AM and DAPT groups. Although there are no recommendations to monitor which are 72-125 U for the COLtest, 57-113 U for the ADP test and 71-115 U for the ASPItest. In our opinion, this difference may be related to the delay in performing the assay (3-4 hours). According to current guidelines, such a delay is acceptable, though we nevertheless currently perform the aggregation tests within 2 hours of blood collection. Interestingly, the reference range for a crucial variable in this study – collagen-induced platelet aggregation (52-117 U) – does not differ significantly from the previous observations by Peerschke et al., who

**Figure 1.** Differences in collagen-, ADP- and AA-induced platelet aggregation between the AM and DAPT groups.

**Figure 2.** Comparison of platelet reactivity inhibition for different agonists (AA, ADP and collagen) in patients on AM or DAPT. The vertical line in the graph indicates the lower values of the reference ranges.
antiplatelet therapy after coronary interventions, an evaluation performed on selected groups to confirm platelet inhibition during antiplatelet therapy is advisable.

In our study, we have observed higher efficacy of dual antiplatelet therapy (DAPT) compared to single antiplatelet therapy (AM) (Fig. 1). The better inhibition of arachidonic acid-induced platelet aggregation in the DAPT patients using 75 mg of ASA compared to AM patients using 150 mg of ASA is likely the result of synergism between the two drugs. This effect has been well described in the literature [27, 28], and our data correspond with the results reported by other authors. However, it is worth noting that incomplete inhibition of collagen-induced platelet aggregation did occur in both study groups. Therefore, it seems reasonable to consider introducing a new treatment option, for example, adding a third antiplatelet drug, such as an antagonist of collagen receptors.

To date, two new antiplatelet drugs – Revacept and glenzocimab (both GPVI antagonists) – have successfully completed phase II clinical trials without reports of bleeding-related side effects [29].

CONCLUSION

To sum up, considering the fact that platelet aggregation in response to collagen in patients receiving antiplatelet therapy after CABG is not completely inhibited, the use of dedicated agonists such as ADP or arachidonic acid for monitoring the effectiveness of antiplatelet therapy is insufficient. The results of this study prompt further investigation into new antiplatelet drugs that will block collagen receptors.

REFERENCES


