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Regular Article

Evaluation of the platelet count drop method for assessment of platelet function in comparison with "gold standard" light transmission aggregometry

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ABSTRACT

Introduction: Hyporesponsiveness to antiplatelet agents has been linked to an increased risk of major adverse cardiovascular events. However, light transmission aggregometry (LTA), the gold standard methodology for assessing platelet function, requires expertise and is labour-intensive, which render its use in clinical settings impractical. We assessed whether platelet count drop (PCD), a technique widely available in any haematology laboratory, could replace LTA in testing for inhibition of platelet aggregation induced by antiplatelet agents.

Materials and methods: One hundred and sixty-one coronary artery disease patients taking aspirin alone and 91 patients taking a combination of aspirin and clopidogrel were enrolled. Platelet aggregation was measured by LTA and PCD stimulated with 1.6 mM of arachidonic acid (AA) for aspirin and 5 and 20 μM of adenosine diphosphate (ADP) for clopidogrel.

Results: Correlation between AA-induced LTA and PCD was inexistent ($r = -0.043$, $p = 0.587$), while correlation between ADP-induced LTA and PCD was low ($r = 0.374$, $p < 0.0001$ for ADP 5 μM and $r = 0.402$, $p < 0001$ for ADP 20 μM). PCD, whether stimulated with AA or ADP, overestimated platelet aggregation as assessed by LTA, by 13-18%. The wide 95% limits of agreement suggest that the assays can disagree significantly in individual patients.

Conclusions: Although the PCD method is widely available in non-specialized laboratories, our results demonstrate that there is poor correlation with the current gold standard, i.e. LTA. Thus, PCD should not be used in replacement of LTA to assess antiplatelet responsiveness.

Introduction

The use of antiplatelet drugs in patients suffering from coronary artery disease (CAD) has become standard practice [1,2]. Their daily administration significantly reduces the risk of acute thrombotic events [3]. However, platelet responses to antiplatelet agents are not equal in all individuals. Several studies have shown that an important proportion of patients display normal platelet aggregation despite daily therapy with aspirin [4] or clopidogrel [5], and hyporesponsiveness to either agent has been associated with an increased risk of acute ischemic events [6–10]. To identify hyporesponsive individuals at risk of recurrent thrombotic events, and to potentially be able to offer antiplatelet therapy tailored to individual needs, accurate assessment of platelet function is required.

Of the platelet function assays available, light transmission aggregometry (LTA), which measures luminosity as aggregation occurs in agonist-stimulated platelet-rich plasma (PRP), is considered by most as the current gold standard [11]. The technique has been used extensively in the last 50 years, and results have been shown to correlate with adverse thrombotic events [11]. It remains to this day the most widely used assay in the assessment of platelet function, despite its requirement for specialized equipment and sample manipulation by a highly-trained technician. Because it is time- and labor-intensive, LTA remains restricted to specialized laboratories, and its routine use in a clinical setting is unlikely.

The platelet count drop method (also called platelet count ratio or impedance platelet counting) assesses platelet aggregation as a ratio of platelet counts before and after the addition of an agonist in whole blood, using a standard electrical impedance cell counter. As platelets

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; CAD, coronary artery disease; EDTA, ethylenediamine tetraacetic acid; LTA, light transmission aggregometry; PCD, platelet count drop.

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Table 1
Demographic characteristics of study population.

Parameter	Aspirin alone (n = 161)	Aspirin and clopidogrel (n = 91)
Age, years	66.3 ± 10.5	61.2 ± 8.4
Male gender, n (%)	122 (74.8%)	75 (82.4%)
BMI, kg/m^2	27.4 ± 4.6	27.8 ± 4.0
Platelet count, 10^9/L	228 ± 55	237 ± 56
Active smoking, n (%)	26 (16.1%)	19 (20.9%)
Dyslipidemia, n (%)	132 (82.0%)	82 (90.1%)
Hypertension, n (%)	119 (73.9%)	61 (67.0%)
Positive family history of CAD, n (%)	86 (53.4%)	57 (62.6%)
Prior unstable angina, n (%)	73 (45.3%)	49 (53.8%)
Prior myocardial infarction, n (%)	98 (60.9%)	24 (26.4%)

aggregate, they exceed the threshold for platelet size identification and are no longer counted as individual platelets, potentially allowing for an easy, fast and widely available method to estimate platelet aggregate formation [12,13].

We performed this study to assess whether PCD, a technique widely available in any haematology laboratory, could replace LTA in testing for inhibition of platelet aggregation induced by antiplatelet agents.

Materials and methods

Patients

One hundred and sixty-one CAD patients taking aspirin alone and 91 patients taking a combination of aspirin and clopidogrel were enrolled, as previously described [14,15]. Patients taking aspirin (80 or 325 mg daily) were recruited from the outpatient cardiology clinic, and patients on the combination of aspirin (80 mg daily) and varying doses of clopidogrel (loading dose of 300 or 600 mg, 24 hours before testing, or 7-daily maintenance dose of 75 or 150 mg prior to angiography) were recruited from the pre-angiography clinic at Hôpital du Sacré-Coeur de Montréal, Canada. Exclusion criteria included: acute coronary syndrome or revascularization within the last 6 months; concurrent ingestion of nonsteroidal anti-inflammatory drugs (NSAID, including COX-2 selective anti-inflammatory drugs), ticlopidine, dipyridamole, warfarin, or acenocoumarol; self-reported use of non-prescription NSAID or drugs containing aspirin in the 10 days preceding enrolment; alcohol or drug abuse; major surgical procedure within 1 month of enrolment; platelet count outside the 100 to 450 × 10^9/L range; hematocrit <25% or hemoglobin <100 g/L; and chronic renal failure requiring dialysis. This study complies with the Declaration of Helsinki, and was approved by the institutional Scientific and Ethics Review Board. All patients gave written informed consent for participation.

Blood sampling and assessment of platelet aggregation

The first 2 mL of blood, drawn by venipuncture through a 21-gauge needle, were discarded. Blood was then drawn into evacuated tubes containing 3.2% sodium citrate. All blood samples were processed within 2 hours of collection. All analyses were performed by a single highly trained and experienced technician.

Light transmission aggregometry

Platelet aggregation using LTA was considered as gold standard in this study [11]. PRP was obtained by centrifugation of citrated whole blood for 10 minutes at 1000 rpm (89 g). Platelet-poor plasma (PPP) was obtained by centrifugation of the remaining blood for 10 minutes at room temperature at 3000 rpm (805 g). Platelet count in the PRP varied from 250 to 450 × 10^9/L. Aggregation was measured at 37 °C with a ChronoLog Aggregometer (540 model, Havertown, PA, USA) after stimulation with an agonist, using PPP as reference. Aggregation curves were recorded for 5 min and analyzed according to international standards.

The agonists used were 1.6 mM (0.5 mg/ml) of arachidonic acid (AA) for assessment of aspirin response, as well as 5 and 20 μM of adenosine diphosphate (ADP) for assessment of clopidogrel response.

Platelet count drop

This method assesses platelet aggregation through classic impedance platelet counting. Using a Coulter® A^C•T Series Analyzer (Beckman Coulter Inc., Fullerton, CA, USA), platelets were counted in 12 μL of fresh citrated whole blood before and after 5 minutes of gentle rocking following the addition of the agonist. Residual platelet aggregation was calculated as follows: % aggregation = (baseline platelet count – post-agonist platelet count)/(baseline platelet count) × 100.

As for LTA, the agonists used were 1.6 mM (0.5 mg/ml) of AA for assessment of aspirin response, and 5 and 20 μM of ADP for assessment of clopidogrel response. To minimize variation in the methods by the use of different agonists, we prepared the reagents in our lab for both the LTA and PCD methods to be used simultaneously for the same patient.

Definitions of inadequate platelet response to antiplatelet agents

Inadequate platelet response to aspirin was defined as AA-induced platelet aggregation ≥20%, as this level has been associated with adverse thrombotic events [6,16].

Inadequate platelet response to clopidogrel was defined as ADP-induced platelet aggregation ≥50% or ≥70%, as both definitions were shown to predict adverse thrombotic events [17–19].

Sample size and statistical analysis

Sample size was not calculated a priori. Post-hoc calculations showed that the study had a power of 80% with a two-sided α-value of

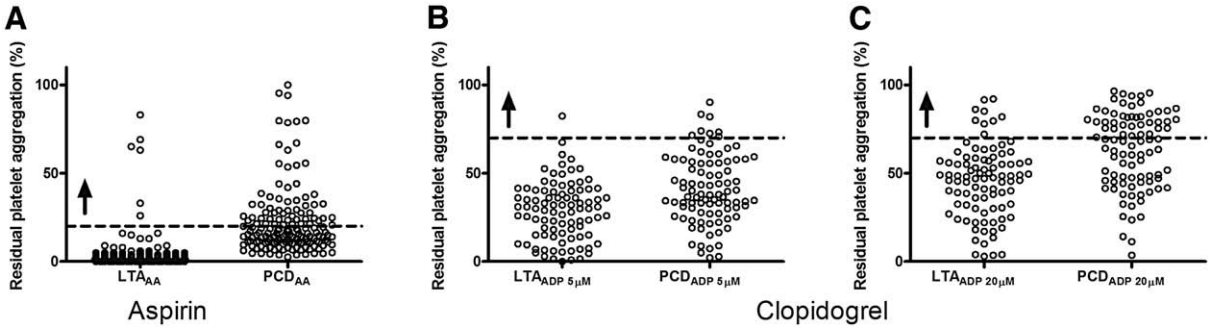

Fig. 1. Platelet aggregation data. A) Aspirin response evaluated by 1.6 mM arachidonic acid as agonist. B) Clopidogrel response evaluated by 5 μM adenosine diphosphate as agonist. C) Clopidogrel response evaluated by 20 μM adenosine diphosphate as agonist. The dotted line represents the cut-off of nonresponsiveness. The arrow indicates the zone within which patients are considered nonresponders. AA: arachidonic acid; ADP: adenosine diphosphate; LTA: light transmission aggregometry; PCD: platelet count drop.