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Response to antiplatelet therapy is independent of endogenous thrombin generation potential

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ABSTRACT

Background: Thrombin is the most potent platelet activator, and achieves rapid platelet activation even in the presence of antiplatelet therapy. Since activated platelets respond stronger to additional stimuli, the extent of endogenous thrombin generation may in part be responsible for the reported response variability to aspirin and clopidogrel therapy.
Patients and methods: Thrombin generation potential was measured with a commercially available assay, and platelet reactivity was assessed with the vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay, light transmission aggregometry (LTA), the VerifyNow aspirin and P2Y12 assays, and multiple electrode aggregometry (MEA) in 316 patients on dual antiplatelet therapy undergoing angioplasty and stenting.
Results: Peak thrombin, the lag phase and the area under the curve of thrombin generation correlated poorly with on-treatment platelet reactivity by all test systems. High on-treatment residual platelet reactivity (HRPR) in response to arachidonic acid was seen in 33 (10.5%), 41 (13%), and 79 (25.7%) patients by LTA, the VerifyNow aspirin assay, and MEA, respectively. HRPR in response to adenosine diphosphate was seen in 150 (48.1%), 48 (15.3%), 106 (33.7%), and 118 (38.3%) patients by the VASP assay, LTA, the VerifyNow P2Y12 assay, and MEA, respectively. Peak thrombin generation did not differ between patients without and with HRPR by the VASP assay, LTA, the VerifyNow P2Y12 assay and MEA. In the VerifyNow aspirin assay, patients without HRPR had higher peak thrombin generation than patients with HRPR ($p = 0.01$). Finally, patients without and with high peak thrombin generation exhibited similar on-treatment platelet reactivity by all test systems, and high peak thrombin generation occurred to a similar extent in patients without and with HRPR.
Conclusion: Response to antiplatelet therapy with aspirin and clopidogrel is not associated with thrombin generation potential.

Introduction

Despite the emergence of new antiplatelet drugs, the combination of aspirin and clopidogrel remains the most commonly used dual antiplatelet regimen to prevent stent thrombosis and future adverse ischemic events after angioplasty and stenting for cardiovascular disease [1]. Aspirin acts directly by inhibiting cyclooxygenase-1 and consecutively blocking thromboxane A2 synthesis [2]. Therefore, high on-treatment residual arachidonic acid (AA) inducible platelet reactivity (HRPR AA) occurs rarely and non-adherence to therapy may be its primary cause [3]. Clopidogrel is a prodrug undergoing hepatic metabolism by the cytochrome P450 enzyme system [4]. Thereby, its active metabolite is generated, binds to the platelet P2Y12 receptor, and irreversibly inhibits platelet activation by adenosine diphosphate (ADP). Consequently, the response to clopidogrel is variable and high on-treatment residual ADP inducible platelet reactivity (HRPR ADP) occurs in many clopidogrel-treated patients [5]. In the last years

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; HRPR AA, high on-treatment residual AA inducible platelet reactivity; HRPR ADP, high on-treatment residual ADP inducible platelet reactivity; PCI, percutaneous coronary intervention; PAR, protease-activated receptor; VTE, venous thromboembolism; VASP, vasodilator-stimulated phosphoprotein; LTA, light transmission aggregometry; MEA, multiple electrode platelet aggregometry; PRI, platelet reactivity index; MFI, mean fluorescence intensity; ARU, aspirin reaction units; PRU, P2Y12 Reaction Units; AU, aggregation units; AUC, area under the curve; ACS, acute coronary syndrome.

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numerous reasons for inadequate platelet inhibition by clopidogrel were identified, ranging from advanced age [6], pharmacological interactions [7–10] and different co-morbidities to genetic predispositions [11,12].

Both, HRPR AA and HRPR ADP were repeatedly linked to a significantly increased risk for ischemic events following percutaneous coronary intervention (PCI) [13–15].

Thrombin is the most potent platelet activator. It acts mainly via protease-activated receptors (PAR)-1 and PAR-4, and achieves rapid platelet activation even in the presence of dual antiplatelet therapy with aspirin and clopidogrel or prasugrel [16,17]. Endogenous thrombin generation has been associated with an elevated risk of first and recurrent venous thromboembolism (VTE) [18,19]. However, levels of thrombin generation have not been linked to platelet reactivity, so far. High levels of thrombin generation may lead to general platelet hyper-reactivity. Since previous studies revealed that activated platelets respond stronger to additional stimuli [20,21], we hypothesized that endogenous thrombin generation potential may contribute to poor response to antiplatelet therapy. We therefore investigated the association of thrombin generation potential with residual platelet reactivity to AA and ADP in patients on dual antiplatelet therapy undergoing angioplasty and stenting for cardiovascular disease.

Materials and Methods

Patients

This was a prospective cohort study. The study population comprised 316 patients on dual antiplatelet therapy one day after percutaneous intervention with endovascular stent implantation. The intake of antiplatelet medication was supervised during the hospital stay (1 day before until 1 day after the angioplasty procedure). The compliance to long-term aspirin and clopidogrel therapy was ensured by in depth interviews. All patients received daily aspirin therapy (100 mg/day). Except 71 patients (22.5%) on clopidogrel maintenance therapy, all patients received a loading dose of 300 mg clopidogrel 24 hours prior to intervention (n = 162; 51.3%) or a loading dose of 600 mg clopidogrel on the day of intervention at least 2 hours prior to angioplasty (n = 83; 26.2%) followed by a daily dose of 75 mg clopidogrel. Additionally, we included 15 patients (10 male, 5 female; age 56 years [52 – 65 years]) receiving 100 mg aspirin and 10 mg prasugrel per day. All patients received 40 mg enoxaparin s.c. in the evening after the intervention for thromboprophylaxis. The median time interval between administration of enoxaparin and blood sampling was 14 hours (12 – 15 hours).

Exclusion criteria were a known aspirin, clopidogrel or prasugrel intolerance (allergic reactions, gastrointestinal bleeding), a therapy with vitamin K antagonists (warfarin, phenprocoumon, acenocoumarol), a treatment with heparin within 12 hours before the enrolment, a treatment with ticlopidine, dipyridamol or nonsteroidal antiinflammatory drugs, a family or personal history of bleeding disorders, malignant paraproteinemias, myeloproliferative disorders or heparin-induced thrombocytopenia, severe hepatic failure, known qualitative defects in thrombocyte function, a major surgical procedure within one week before enrollment, a platelet count <100.000 or >450.000/μl and a hematocrit <30%.

The study protocol was approved by the Ethics Committee of the Medical University of Vienna in accordance with the Declaration of Helsinki and written informed consent was obtained from all study participants.

Blood Sampling

Blood was drawn by aseptic venipuncture from an antecubital vein using a 21-gauge butterfly needle (0.8 × 19 mm; Greiner Bio-One, Kremsmünster, Austria) one day after percutaneous intervention. To avoid procedural deviations, all blood samples were taken by the same physician applying a light tourniquet, which was immediately released, and the samples were mixed by gently inverting the tubes. The first 3 ml of blood were discarded to reduce procedurally induced platelet activation. The following aliquots of blood were drawn into a 3.8% sodium citrate Vacuette tube (Greiner Bio-One; 9 parts of whole blood, 1 part of sodium citrate 0.129 M/L) for the vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay, light transmission aggregometry (LTA), and measurement of thrombin generation, into a 3.2% sodium citrate Vacuette tube (Greiner Bio-One; 9 parts of whole blood, 1 part of sodium citrate 0.109 M/L) for the VerifyNow assays, and into a Vacuette tube containing lithium heparin (18 IU/ml) for the determinations by multiple electrode aggregometry (MEA). The time interval between blood sampling and platelet function testing was at least 1 hour and did not exceed 3 hours, all MEA measurements were performed between 1 and 2 hours after blood sampling. Platelet poor plasma for the assessment of thrombin generation was obtained within 1 hour after blood sampling by centrifugation at 2000 ×*g*, and stored at -80 °C until analysis. To avoid investigator-related variations of the results, each of the different tests was performed by just one corresponding operator, who was blinded to the results from the other operators.

Vasodilator Stimulated Phosphoprotein (VASP) Phosphorylation assay

The platelet reactivity index (PRI) was determined according to a standardized flow cytometric assay (Platelet VASP, Diagnostica Stago, Biocytex, Marseille, France) as previously described [22]. In brief, the extent of VASP phosphorylation was measured by geometric mean fluorescence intensity (MFI) values in the presence of PGE1 without (T1) or with ADP (T2). After subtraction of the negative isotypic control values from the corresponding fluorescence values, PRI (%) was calculated according to the following formula:

$$PRI\% = [T1(PGE1) - T2(PGE1 + ADP)/T1(PGE1)] \times 100$$

Results from the VASP assay were available for 312 patients (98.7%).

Light Transmission Aggregometry (LTA)

For LTA, platelet counts were not adjusted as the median platelet count was 208 G/L (176 – 251 G/L) [23,24]. Aggregation was performed using AA (final concentration of 0.5 mg/ml; LTA AA) and ADP (10 μM; LTA ADP; both from Rolf Greiner BioChemica, Flacht, Germany). Optical density changes were recorded photoelectrically for 10 minutes as platelets began to aggregate. Results from LTA AA were available for 313 patients (99.1%), results from LTA ADP were available for 314 patients (99.4%).

VerifyNow Aspirin and P2Y12 Assay

In the VerifyNow aspirin and P2Y12 assays (Accumetrics, San Diego, CA, USA), higher Aspirin Reaction Units (ARU) and higher P2Y12 Reaction Units (PRU) reflect greater AA- and ADP-mediated platelet reactivity, respectively [23,24]. Results from the VerifyNow aspirin assay were available from all patients, results from the VerifyNow P2Y12 assay were available from 315 patients (99.7%).

Multiple Electrode Platelet Aggregometry (MEA)

In whole blood impedance aggregometry, AA (final concentration of 0.5 mM; MEA AA) or ADP (6.4 μM; MEA ADP; both from Verum Diagnostica, Munich, Germany) activated platelets adhered to the electrodes leading to an increase of impedance, which was detected for each sensor unit separately and transformed to aggregation units (AU) that were plotted against time [25]. Results from MEA