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Response to antiplatelet therapy and platelet reactivity to thrombin receptor activating peptide-6 in cardiovascular interventions: Differences between peripheral and coronary angioplasty

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ABSTRACT

Background: The long-term prognosis of patients with peripheral arterial disease (PAD) is significantly worse than the prognosis of coronary artery disease (CAD) patients. Detrimental platelet activation could contribute to the increased rate of adverse cardiovascular events in PAD. We therefore investigated whether response to antiplatelet therapy and thrombin inducible platelet activation differ between patients with best medical therapy undergoing angioplasty and stenting for symptomatic PAD ($n = 166$) or CAD ($n = 104$).

Methods: Adenosine diphosphate (ADP), arachidonic acid (AA) and thrombin receptor activating peptide (TRAP)-6 inducible platelet reactivity was measured by multiple electrode aggregometry (MEA). Platelet surface expression of P-selectin and activated glycoprotein IIb/IIIa (GPIIb/IIIa) in response to ADP, AA, and TRAP-6, and the formation of monocyte-platelet aggregates (MPA) in response to ADP and TRAP-6 were assessed by flow cytometry.

Results: Patients with PAD had significantly higher platelet reactivity in response to ADP and AA by MEA compared to CAD patients. Likewise, the expression of P-selectin and GPIIb/IIIa following stimulation with ADP and AA, and MPA formation in response to ADP were significantly higher in PAD patients than in CAD patients. In response to TRAP-6, patients with PAD showed a significantly increased platelet aggregation by MEA, higher expression of activated GPIIb/IIIa, and more pronounced formation of MPA than CAD patients.

Conclusion: Following angioplasty and stenting, PAD patients exhibit a significantly diminished response to dual antiplatelet therapy and an increased susceptibility to TRAP-6 inducible platelet activation compared to CAD patients.

1. Introduction

Peripheral arterial disease (PAD) and coronary artery disease (CAD) are different manifestations of systemic atherosclerosis. Patients with PAD often have multiple affected vascular beds [1], and the prevalence of CAD in PAD patients ranges from 46% to 71% [2,3]. Adverse cardiac or cerebrovascular events are the most common cause of death in patients with PAD. Recently, it has been shown that the long-term prognosis of patients with PAD undergoing vascular surgery is significantly worse than the prognosis of CAD patients undergoing percutaneous coronary intervention (PCI) [4]. The increase in adverse events in PAD patients may in part be attributable to less intensive risk factor treatment [5]. However, detrimental platelet activation and high platelet reactivity despite dual antiplatelet therapy are considered major reasons of adverse cardiovascular events, and could contribute to the inferior clinical outcome of patients with PAD [6–9]. While dual antiplatelet therapy inhibits arachidonic acid (AA) and adenosine diphosphate (ADP) inducible platelet reactivity after angioplasty and stenting, platelet activation by thrombin may still be maintained [10,11]. We therefore investigated whether response to antiplatelet therapy and residual thrombin receptor activating peptide (TRAP)-6 inducible platelet activation differ between patients with best medical therapy undergoing angioplasty and stenting for symptomatic PAD and CAD.

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2. Methods

2.1. Patients

The study population consisted of 270 patients on dual antiplatelet therapy after percutaneous intervention with endovascular stent implantation. One hundred sixty-six patients were treated with peripheral angioplasty and 104 patients were treated with coronary angioplasty. All patients had received daily aspirin therapy (100 mg/d). Except 50 patients (18.5%) on clopidogrel maintenance therapy, all patients received a loading dose of 300 mg clopidogrel 24 h prior to intervention ($n = 143$; 53%) or a loading dose of 600 mg clopidogrel on the day of intervention at least 2 h prior to angioplasty ($n = 77$; 28.5%) followed by a daily dose of 75 mg clopidogrel. During the angioplasty procedure, PAD patients received 5.000 units of unfractionated heparin, while heparin dosage was weight adjusted in patients undergoing coronary angioplasty and stenting (100 units of heparin/kg). Moreover, all patients received 40 mg enoxaparin s.c. for thromboprophylaxis in the evening after the successful intervention. All patients with symptomatic PAD had intermittent claudication, but no clinical signs of CAD. TASC A, B, C, and D lesions were observed in 23 (13.9%), 101 (60.8%), 38 (22.9%) and 4 (2.4%) PAD patients, respectively. Clinical presentation scenarios of patients with symptomatic CAD were stable angina, unstable angina/non-ST-segment elevation myocardial infarction (UA/NSTEMI), and ST-segment elevation myocardial infarction (STEMI) in 41 (39.4%), 41 (39.4%), and 22 (21.2%) patients, respectively.

Exclusion criteria were a known aspirin or clopidogrel intolerance (allergic reactions, gastrointestinal bleeding), a therapy with vitamin K antagonists (warfarin, phenprocoumon, acenocoumarol), 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.

2.2. Blood sampling

Blood was drawn by clean venipuncture from an antecubital vein using a 21-gauge butterfly needle (0.8 × 19 mm; Greiner Bio-One, Kremsmünster, Austria) one day after the 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 adequately by gently inverting the tubes. After the initial 3 ml of blood had been discarded to reduce procedurally induced platelet activation, blood was 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 flow cytometric analyses, and into a Vacuette tube containing lithium heparin (18 IU/ml) for the determinations by multiple electrode aggregometry (MEA).

2.3. Multiple electrode platelet aggregometry (MEA)

Whole blood impedance aggregometry was performed as previously described [12,13]. After addition of ADP (6.4 μM), AA (final concentration of 0.5 mM), or TRAP-6 (32 μM; all from Verum Diagnostica, Munich, Germany), the adhesion of activated platelets to the electrodes led to an increase of impedance, which was detected for each sensor unit separately and transformed to aggregation units (AU) that were plotted against time.

2.4. Platelet surface expression of P-selectin and activated glycoprotein IIb/IIIa

The expression of P-selectin and the binding of the monoclonal antibody PAC-1 to activated glycoprotein IIb/IIIa (GPIIb/IIIa) were determined in citrate anticoagulated blood, as previously published [14]. In brief, whole blood was diluted in phosphate buffered saline to obtain 20×10^3 platelets and incubated without agonists and after *in vitro* exposure to suboptimal concentrations of ADP (1 μM, Verum Diagnostica, Munich, Germany), AA (80 μM; Diamed, Cressier, Switzerland) or TRAP-6 (5.7 μM; Bachem, Bubendorf, Switzerland) for 10 min. The platelet population was identified by staining with anti-CD42b (clone HIP1, allophycocyanin labeled; Becton Dickinson (BD), San Jose, CA, USA), and expression of activated GPIIb/IIIa and P-selectin were determined by the binding of the monoclonal antibodies PAC-1-fluorescein (BD) and anti-CD62p-phycoerythrin (clone CLB-Thromb6; Immunotech, Beckman Coulter, Fullerton, CA, USA), respectively. After 15 min of incubation in the dark, the reaction was stopped by adding 500 μL PBS and samples were acquired immediately on a FACS Calibur flow cytometer (BD) with excitation by an argon laser at 488 nm and a red diode laser at 635 nm at a rate of 200–600 events per second. Platelets were gated in a side scatter versus FL3 dot plot. A total of 10.000 events were acquired within this gate. The gated events were further analyzed in histograms for FL-1 and FL-2 for PAC-1 and P-selectin, respectively. Standard BD calibrite beads were used for daily calibration of the cytometer.

2.5. Determination of monocyte-platelet aggregates

Monocyte-platelet aggregates (MPA) were identified in citrate anticoagulated blood. In brief, platelet agonists ADP 1.5 μM, TRAP-6 7.1 μM, or HEPES buffer were added to 5 μl whole blood, diluted in 55 μl HEPES-buffered saline. After 10 min, monoclonal antibodies (anti-CD45-peridinin chlorophyll protein (clone 2D1, BD), anti-CD41-phycoerythrin, (clone P2, Immunotech), and anti-CD14-allophycocyanin (clone MφP9, BD)), or istoype-matched controls were added. After 15 min, samples were diluted with FACSlysing solution and at least 10,000 CD45+ events were acquired immediately. Within these events, lymphocytes, granulocytes, and monocytes were identified, based on their CD14 versus side scatter characteristics. Monocytes were identified as CD14+ and the CD45+CD14+ events were subjected to further analyses for CD45+CD41+ and CD45+CD41− events. The percentage of CD14+CD41+ events was recorded.

2.6. Statistical analysis

A sample size calculation was based on the observed mean ± SD (49 ± 22 AU) of MEA in response to ADP in a former population of 80 patients (45 male, 35 female; age 66 years (59–74 years)) receiving dual antiplatelet treatment 24 h after angioplasty and stenting [12]. We calculated that we needed to include 250 patients to be able to detect a 20% relative difference of platelet reactivity by MEA between patients with PAD and patients with CAD with a power of 93% (using a two-sided alpha level of 0.05). To compensate for potential technical problems we included 20 additional patients.

Statistical analysis was performed using the Statistical Package for Social Sciences (IBM SPSS version 19, Armonk, New