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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

York, USA). Median and interquartile range of continuous variables are shown. Categorical variables are given as number (%). We performed Mann Whitney *U* tests to detect differences of MEA, and flow cytometric parameters of platelet activation between patients with PAD and CAD. Multivariate regression analysis was used to adjust for differences in patient characteristics. The chi-square test was used to detect differences in categorical variables. Spearman rank correlation was used to assess correlations between different platelet activation markers. Two-sided *p*-values <0.05 were considered statistically significant.

3. Results

Clinical, laboratory, genetic, and procedural characteristics of the overall patient population ($n = 270$), of patients undergoing peripheral angioplasty ($n = 166$) and of patients undergoing coronary angioplasty ($n = 104$) are given in Table 1. Genotyping for loss-of-function polymorphisms of cytochrome 2C9 and cytochrome 2C19 was available for 247 patients (91.5%). As expected, diabetes was more frequent in patients with PAD, and the use of beta blockers was more common in patients with CAD. Further, angiotensin converting enzyme inhibitors and angiotensin receptor blockers were used more frequently in patients with CAD. The remaining patient characteristics did not differ significantly between PAD and CAD patients. Results from MEA were available from all patients, activated GPIIb/IIIa was available from 266 patients (98.5%), and MPA formation was available from 228 patients (84.4%).

Baseline values of P-selectin expression, activated GPIIb/IIIa, and MPA formation without addition of platelet agonists were significantly higher in PAD patients (Table 2).

In a first step, we evaluated the response to aspirin and clopidogrel in both subgroups. Thereby, patients with PAD had significantly higher platelet reactivity in response to ADP and AA by MEA compared to CAD patients (Fig. 1A, B). Further, the expression of P-selectin and activated GPIIb/IIIa after stimulation with ADP and AA, and MPA formation in response to ADP were significantly higher in PAD patients than in CAD patients (Table 2).

In a second step, we assessed the susceptibility of PAD and CAD patients to stimulation of protease activated receptor (PAR)-1 by TRAP-6. In response to TRAP-6, patients with PAD showed a significantly increased platelet aggregation by MEA compared to CAD patients (Fig. 1C). Further, in patients with PAD, platelet surface expression of activated GPIIb/IIIa and the formation of MPA after stimulation with TRAP-6 were significantly more pronounced than in CAD patients (Table 2).

After adjustment for diabetes and differences in drug therapy by multivariate regression analyses, PAD remained a significant predictor of increased ADP, AA, and TRAP-6 inducible platelet reactivity by MEA (all $p < 0.05$; Table 3 A–C).

Table 2
Platelet surface expression of P-selectin and activated glycoprotein IIb/IIIa (GPIIb/IIIa) without agonists, and in response to adenosine diphosphate (ADP), arachidonic acid (AA) or thrombin receptor activating peptide (TRAP)-6, and formation of monocyte-platelet aggregates (MPA) without agonists, and in response to ADP or TRAP-6 in patients undergoing peripheral (PAD) or coronary (CAD) angioplasty.

	PAD	CAD	*p*-value
P-selectin (MFI)	3.4 (2.9–4.2)	3 (2.6–3.8)	0.005
P-selectin ADP (MFI)	13.5 (8.3–21.7)	11.4 (6.2–18.4)	0.03
P-selectin AA (MFI)	6.9 (4.7–10.5)	5.3 (4–7.6)	0.001
P-selectin TRAP-6 (MFI)	57.4 (30.5–102.5)	54.2 (23.4–90.1)	0.2
GPIIb/IIIa (MFI)	2.9 (2.4–3.6)	2.6 (2.3–3.3)	0.02
GPIIb/IIIa ADP (MFI)	11.5 (7.4–17.3)	8 (6.1–15.7)	0.01
GPIIb/IIIa AA (MFI)	6.1 (4.2–9.8)	4.5 (3.5–7.5)	0.001
GPIIb/IIIa TRAP-6 (MFI)	4.6 (3.2–7)	3.8 (2.9–5.6)	0.008
MPA (%)	23.8 (15.8–34.4)	20.3 (12.4–26.8)	0.02
MPA ADP (%)	52.2 (39.3–66.5)	46.2 (33–56.9)	0.02
MPA TRAP-6 (%)	76.3 (60–86.5)	68.2 (51.6–84.2)	0.02

Continuous data are given as median (interquartile range).
AU, aggregation units; MFI, mean fluorescence intensity.

Table 1
Clinical, laboratory, genetic, and procedural characteristics of the overall study population, and of patients undergoing peripheral (PAD) or coronary angioplasty (CAD).

Characteristics	Overall ($n = 270$)	PAD ($n = 166$)	CAD ($n = 104$)	*p*-value
Demographics				
Age, years	65 (58–74)	66 (58–73)	64 (55–75)	0.5
Male sex	177 (65.6)	103 (62)	74 (71.2)	0.1
BMI, kg/m^2	26.8 (24.4–29.6)	26.7 (24.5–29.4)	27.6 (24.3–29.7)	0.5
Medical history				
Hypertension	242 (89.6)	152 (91.6)	90 (86.5)	0.2
Hypercholesterolemia	250 (92.6)	156 (94)	94 (90.4)	0.3
Diabetes mellitus	88 (32.6)	63 (38)	25 (24)	0.02
Active smoking	115 (42.6)	74 (44.6)	41 (39.4)	0.4
Laboratory data				
Serum creatinine, mg/dl	1 (0.9–1.2)	1 (0.9–1.2)	1 (0.9–1.1)	0.3
C-reactive protein, mg/dl	1 (0.4–2.1)	1.1 (0.4–1.9)	0.8 (0.4–2.7)	0.9
Genetics				
CYP2C9 LOF	39 (15.8)	21 (14.2)	18 (18.2)	0.4
CYP2C19 LOF	75 (30.4)	47 (31.8)	28 (28.3)	0.6
Procedure				
Stent implantation	270 (100)	166 (100)	104 (100)	1
Number of stents/patient	1 (1–2)	1 (1–2)	1 (1–2)	0.4
Medication pre-intervention				
Aspirin	270 (100)	166 (100)	104 (100)	1
Clopidogrel	270 (100)	166 (100)	104 (100)	1
Clopidogrel loading dose	220 (81.5)	138 (83.1)	82 (78.8)	0.4
Statins	257 (95.2)	156 (94)	101 (97.1)	0.2
ACE inhibitors/ARB	238 (88.1)	140 (84.3)	98 (94.2)	0.01
Beta blockers	191 (70.7)	94 (56.6)	97 (93.3)	<0.01
Proton pump inhibitors	141 (52.2)	79 (47.6)	62 (59.6)	0.1

Continuous data are shown as median (interquartile range). Dichotomous data are shown as *n* (%).
BMI, body mass index; CYP2C9 LOF, loss-of-function polymorphisms of cytochrome 2C9; CYP2C19 LOF, loss-of-function polymorphisms of cytochrome 2C19; ACE inhibitors, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers.

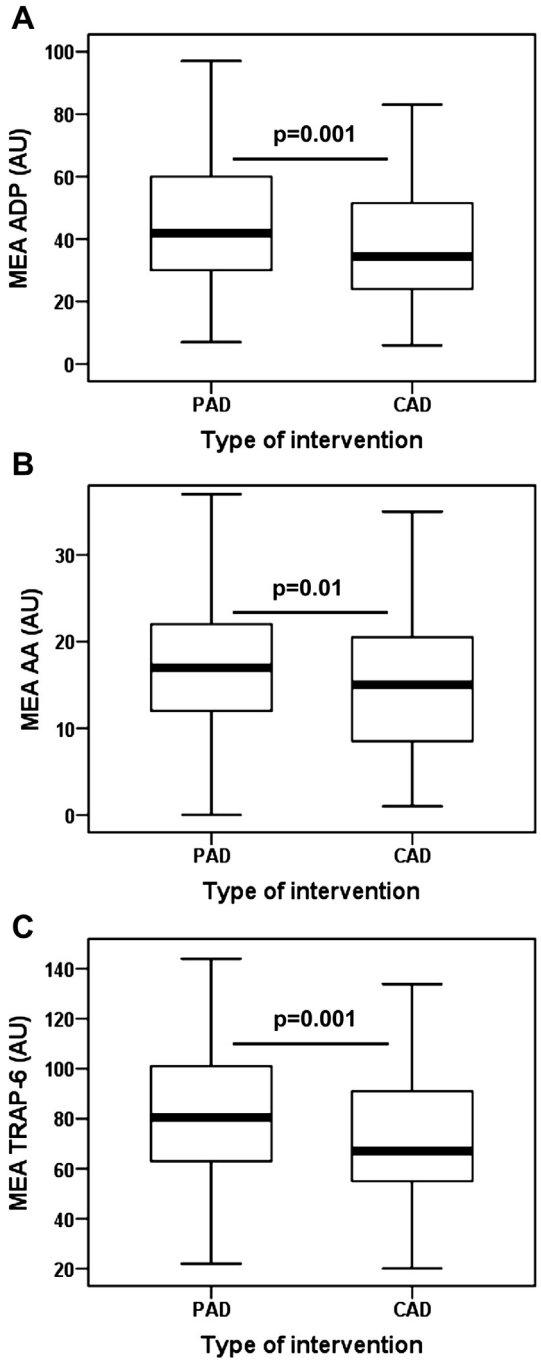

Fig. 1. Platelet aggregation in response to (A) adenosine diphosphate (ADP), (B) arachidonic acid (AA), and (C) thrombin receptor activating peptide (TRAP)-6 by multiple electrode aggregometry (MEA) in patients undergoing peripheral (PAD) and coronary (CAD) angioplasty.

Except activated GPIIb/IIIa and MPA formation without the addition of agonists, all parameters of platelet activation correlated significantly with each other (Table 4).

Residual platelet reactivity to ADP and TRAP-6 by MEA, and MPA formation *in vivo*, in response to ADP and in response to TRAP-6 were significantly higher in female compared to male patients (all $p < 0.05$).

In PAD patients, the TASC grade was not associated with parameters of platelet reactivity and activation (all $p > 0.05$). In patients with CAD, platelet reactivity and activation did not differ significantly between patients with stable angina ($n = 41$) and ACS ($n = 63$), respectively (all $p > 0.05$). The formation of MPA in response to TRAP-6 was significantly more pronounced in patients with STEMI ($n = 22$) compared to patients with UA/NSTEMI ($n = 41$; $p = 0.04$). The remaining parameters of platelet reactivity and activation were similar between patients with STEMI and patients with UA/NSTEMI (all $p > 0.05$).

Table 3A
Regression coefficients (B), 95% confidence intervals (CI), and *p*-values (*p*) of multivariate regression analysis of peripheral arterial disease (PAD), diabetes, use of beta blockers, and use of angiotensin converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARB) for adenosine diphosphate inducible platelet reactivity by multiple electrode aggregometry.

Variable	B	95% CI	*p*
PAD	7	1.5–12.5	0.01
Diabetes	−2	−7.2 to 3.3	0.5
Beta blockers	−4.5	−10.3 to 1.4	0.1
ACE inhibitors/ARB	5.9	−1.8 to 13.6	0.1

4. Discussion

To the best of our knowledge, our study is the first to compare response to antiplatelet therapy and susceptibility to PAR-1 stimulation between patients undergoing peripheral and coronary angioplasty. We found significantly higher residual platelet reactivity and parameters of platelet activation in response to ADP and AA in PAD patients compared to CAD patients. Further, we report increased PAR-1 mediated platelet reactivity in patients treated with peripheral angioplasty.

In a previous study, showing a worse long-term prognosis for patients with PAD compared to CAD patients, the increase in adverse events in PAD patients was partly attributable to less intense risk factor management in these patients [4]. Therefore, we included only patients receiving best medical therapy in both treatment groups.

We selected specific agonists to determine platelet reactivity: Arachidonic acid to estimate cyclooxygenase inhibition, ADP to assess residual responsiveness of P2Y12, and TRAP-6 to study thrombin inducible platelet activation through PAR-1. These agonists were applied in two different assays, as assay-specific responsiveness may bias the results. We chose MEA to assess residual platelet reactivity during dual antiplatelet therapy. MEA is based on the principle of impedance aggregometry and high platelet reactivity by MEA despite clopidogrel therapy was recently associated with the occurrence of stent thrombosis after coronary angioplasty [9]. Second, platelet activation in whole blood was estimated by flow cytometry, which has the advantage to require minimal amounts of samples. Thereby we assessed alpha granule secretion by determination of P-selectin expression, which plays a significant role in atherosclerosis [15,16]. The fibrinogen binding site on GPIIb/IIIa was determined as it is exposed on platelets

Table 3B
Regression coefficients (B), 95% confidence intervals (CI), and *p*-values (*p*) of multivariate regression analysis of peripheral arterial disease (PAD), diabetes, use of beta blockers, and use of angiotensin converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARB) for arachidonic acid inducible platelet reactivity by multiple electrode aggregometry.

Variable	B	95% CI	*p*
PAD	4	0.4–7.6	0.03
Diabetes	0.5	−3 to 3.9	0.8
Beta blockers	0.8	−3.1 to 4.6	0.7
ACE inhibitors/ARB	0.5	−4.6 to 5.6	0.9

Table 3C
Regression coefficients (B), 95% confidence intervals (CI), and *p*-values (*p*) of multivariate regression analysis of peripheral arterial disease (PAD), diabetes, use of beta blockers, and use of angiotensin converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARB) for thrombin receptor-activating peptide-6 inducible platelet reactivity by multiple electrode aggregometry.

Variable	B	95% CI	*p*
PAD	9.8	2.6–17.1	0.01
Diabetes	−0.6	−7.6 to 6.3	0.9
Beta blockers	−5.9	−13.6 to 1.9	0.1
ACE inhibitors/ARB	4.7	−5.6 to 15	0.4

undergoing shape change [17]. MPA formation reflects the interaction of activated platelets with monocytes, and circulating MPA are considered an even more sensitive marker of platelet activation than P-selectin in several pathophysiological circumstances, including myocardial infarction [18–20].

The larger extent of atherosclerosis in PAD patients compared to patients with CAD may influence ongoing platelet activation and response to platelet inhibitory treatment. Our data indicate that patients undergoing peripheral angioplasty have a weaker response to aspirin and clopidogrel compared to patients treated with PCI. Low response to antiplatelet therapy has been reported to be a strong risk factor for future adverse ischemic events after angioplasty and stenting [7–9,21]. Further, PAD patients showed increased platelet responsiveness to stimulation of PAR-1. Thrombin is the most potent platelet activator, and acts via four receptors, PAR-1, PAR-4, glycoprotein Ibα and GPV [22–27]. Abundant thrombin generation and consecutive platelet activation are considered major reasons for adverse ischemic events in patients with cardiovascular disease. Both, poor response to antiplatelet therapy and increased susceptibility to PAR-1 mediated platelet activation may therefore in part be responsible for the worse long-term prognosis of PAD patients compared to patients with CAD [4]. As mentioned above, less intensive risk factor management and the accumulation of various comorbidities (e.g. chronic kidney disease, diabetes) may also play an important role for the increased rate of adverse cardiovascular events in PAD [4,5]. However, since platelet activation with subsequent thrombus formation is an integral part in the development of ischemic events, detrimental platelet activation may be one important factor.

Recently, two studies on the thrombin receptor antagonist vorapaxar in patients with acute coronary syndromes (ACS) and in patients with stable atherosclerosis were published [28,29]. In both trials, the inhibition of PAR-1 with vorapaxar significantly reduced the risk of cardiovascular death and ischemic events, but increased the risk of major bleedings, including intracranial hemorrhage. In the ATLAS ACS 2-TIMI 51 trial, the inhibition of thrombin generation by rivaroxaban in patients with a recent ACS yielded promising results regarding the reduction of adverse ischemic events [30]. However, similar to vorapaxar, rivaroxaban increased the rate of major bleedings and intracranial hemorrhage. Consequently, it remains unclear which patient populations are in need of more intense antiplatelet therapy. The assessment of platelet reactivity and platelet activation parameters may allow the identification of patient subgroups, who benefit from additional antithrombotic therapy while being at an acceptable bleeding risk. Our findings suggest that patients undergoing peripheral angioplasty may be candidates for alternative antiplatelet regimens. In detail, PAD patients may benefit from selective inhibition of thrombin-induced platelet activation using PAR antagonists, which may additionally provide a safer pharmaceutical option by sparing some of the hemostatic functions of thrombin [31]. This is supported by the subanalysis of PAD patients in the TRA2°P-TIMI 50 trial, which revealed a significantly reduced risk of acute limb ischemia in patients treated with the PAR-1 antagonist vorapaxar [32].

Table 4
Correlation coefficients (*r*) and *p*-values (*p*) of correlations between P-selectin expression, activated glycoprotein IIb/IIIa (GPIIb/IIIa) and monocyte-platelet aggregate formation (MPA) without and with the addition of agonists.

Parameters	*r*	*p*
P-selectin – GPIIb/IIIa	0.59	<0.001
P-selectin – MPA	0.38	<0.001
GPIIb/IIIa – MPA	0.11	0.09
P-selectin with ADP – GPIIb/IIIa with ADP	0.69	<0.001
P-selectin with ADP – MPA with ADP	0.58	<0.001
GPIIb/IIIa with ADP – MPA with ADP	0.26	<0.001
P-selectin with AA – GPIIb/IIIa with AA	0.62	<0.001
P-selectin with TRAP-6 – GPIIb/IIIa with TRAP-6	0.47	<0.001
P-selectin with TRAP-6 – MPA with TRAP-6	0.61	<0.001
GPIIb/IIIa with TRAP-6 – MPA with TRAP-6	0.34	<0.001

ADP, adenosine diphosphate; AA, arachidonic acid; TRAP-6, thrombin receptor-activating peptide-6.

Interestingly, female patients had significantly higher platelet reactivity to ADP and TRAP-6 by MEA, and showed a more pronounced MPA formation *in vivo*, in response to ADP and in response to TRAP-6 than male patients. This is in line with previous observations, which reported increased platelet reactivity in women during dual antiplatelet therapy [33,34].

Heparin may influence platelet reactivity. However, in consideration of the elimination half-life of unfractionated heparin of 1.5 h [35], it is unlikely that platelet reactivity measurements 24 h after the angioplasty procedure were influenced by heparin dosage during percutaneous intervention.

The median time interval between the administration of low molecular weight heparin and blood sampling was 14 h. Further, all patients received only a low dose of 40 mg enoxaparin with an elimination half-life of 4.1 h, and the median serum creatinine was 1 mg/dl (0.9–1.2 mg/dl) [36]. Therefore, a significant influence of low molecular weight heparin on platelet reactivity is improbable in our study population.

A limitation of our study is the lack of clinical outcome data. Further, systemic atherosclerosis affects more or less arteries of all vascular beds. Consequently, we cannot rule out that patients with PAD and CAD also had other manifestations of asymptomatic atherosclerosis. Likewise, we have no proof that patients with PAD did not have CAD. Thus, only major clinical symptoms guided the differentiation between CAD and PAD. Finally, the timing of testing may affect the results. However, all measurements were performed one day after the percutaneous intervention with stent implantation in all patients. Consequently, the impact of the time factor was similar in all patients.

In 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. Large clinical trials are warranted to clarify whether thrombin receptor antagonists or inhibition of thrombin generation are beneficial in these patients.

Disclosures

None.

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