Human platelet protease-activated receptor-1 responsiveness to thrombin related to P2Y12 inhibition

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Dual antiplatelet therapy with aspirin and adenosine diphosphate (ADP) receptor inhibitors significantly improves the outcome of patients with stable coronary heart disease. However, abundant thrombin generation, which is not influenced by this dual antiplatelet therapy, is a major reason for recurrent thromboembolic disease in these patients. We, therefore, assessed in a hypothesis generating study in patients with stable coronary artery disease specifically the relation of responsiveness of the platelet thrombin receptor protease-activated receptor (PAR)-1 to the magnitude of the inhibition of the ADP receptor. PAR-1 regulation was studied prospectively in 86 consecutive patients with stable coronary artery disease treated with aspirin and clopidogrel (67 patients) or prasugrel (19 patients) and correlated the data to ADP inducible platelet reactivity by impedance aggregometry. PAR-1 expression did not differ between patients on aspirin and clopidogrel vs patients on aspirin and prasugrel ($P > 0.5$). PAR-1 levels were correlated to P-selectin expression ($P < 0.0001$). The higher the PAR-1 expression the more profound was the *in vitro* thrombin-inducible platelet activation. However, neither *ex vivo* PAR-1 expression nor *in vitro* thrombin-inducible PAR-1 were correlated to ADP-inducible platelet aggregation ($P > 0.5$).

Thus, like in a real life scenario, patients with stable ischemic heart disease on dual antiplatelet therapy may express high levels of PAR-1, which are associated with profound thrombin-inducible platelet activation. This responsiveness cannot be predicted by the magnitude of ADP responsiveness. (Translational Research 2013;161:414–420)

Abbreviations: ADP = adenosine diphosphate; AU = aggregation units; CAD = coronary artery disease; GP = glycoprotein; MEA = multiple electrode impedance aggregometry; PAR = protease-activated receptor; PCI = percutaneous coronary intervention

Thrombotic events are a primary cause of morbidity and mortality following percutaneous coronary intervention (PCI) and stent placement despite aspirin and thienopyridine treatment.[1] Platelet activation may occur through pathways that are not affected by cyclooxygenase or P2Y12 inhibitors. Therefore, alternative therapeutic platelet inhibitors are currently investigated.[2]

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AT A GLANCE COMMENTARY

Background

Abundant thrombin generation, which is not influenced by therapy with aspirin and P2Y12 inhibitors, is a major reason thromboembolism in patients with stable coronary heart disease. We assessed the relation of responsiveness of the platelet thrombin protease-activated receptor (PAR)-1 to that of P2Y12 inhibition.

Translational Significance

In vivo levels of the thrombin receptor were correlated with *in vivo* platelet activation. The higher the expression of the thrombin receptor the more profound was the *in vitro* thrombin-inducible platelet activation. However, this responsiveness to thrombin was not predicted by the magnitude of inhibition of the P2Y12 receptor.

Abundant thrombin generation is considered the major reason for recurrent acute thromboembolic events in patients on dual antiplatelet therapy. Thrombin activates platelets via 4 distinct receptors, protease-activated receptors (PAR)-1 and -4, and glycoprotein (GP) Ibα and GP V.[3-8] There is strong evidence that the PAR-1 platelet activation pathway is clinically significant. For example, platelet PAR-1 modulation has been seen in patients with stroke.[5] Furthermore, recent data have demonstrated that despite effective dual platelet inhibition, thrombin generation during PCI can activate platelets.[9,10] Thrombosis, leukocyte chemotaxis, smooth muscle cell proliferation, and migration may ensue and stimulate vascular remodeling.

Inhibition of P2Y12 is associated with a reduced PAR-1 mediated aggregation,[11,12] but the receptor's regulation by thrombin under P2Y12 inhibition has not been addressed so far. To simulate a real life scenario, we studied relations of PAR-1 regulation with the efficacy of P2Y12 inhibition, and with platelet activation in patients with stable coronary artery disease (CAD) on aspirin and thienopyridine treatment.

METHODS

Patients. The study complied with the Declaration of Helsinki, was approved by the Ethics Committee of the Medical University of Vienna, and all patients and controls gave written informed consent. We prospectively studied if PAR-1 regulation is maintained despite therapeutic inhibition of the P2Y12 receptor. The prospective study was designed to test the hypothesis that PAR-1 regulation is maintained despite therapeutic inhibition of the platelet P2Y12 adenosine diphosphate (ADP) receptor. Blood samples were obtained from 86 consecutive patients with angiographically proven stable CAD (Table I). Of these, 68 patients had been treated with stents (50 patients were on clopidogrel, 18 patients on prasugrel), and the other 18 patients were treated conservatively (17 on clopidogrel, 1 on prasugrel). Blood was drawn in a stable condition, after coronary angiography and interventions had been successfully accomplished, and after patients were on continuous therapy with aspirin (100 mg daily) and either clopidogrel (75 mg daily) or prasugrel (10 mg daily) for at least 5 days (range 5–10 days). Patients who had been pretreated with thrombolytic therapy or platelet GP IIb/IIIa antagonists were excluded.

Table I. Clinical, laboratory, and procedural characteristics of the study population

Characteristics	n = 86
Age, y	63 (51; 72)
Male sex	67 (77.9%)
BMI, kg/m^2	28.07 (−24.61; 31.20)
Medical history	
History of CAD	30 (34.9%)
Hypertension	60 (69.8%)
Hypercholesterolemia	58 (67.4%)
Diabetes mellitus	26 (30.2%)
Active smoking	52 (60.5%)
Laboratory data	
Platelet count, $\times 10^9$/L	215 (189; 264)
Serum creatinine, mg/dL	1.02 (0.89; 1.2)
C-reactive protein, mg/dL	0.94 (−0.31 to 1.76)
Baseline medication	
Aspirin (100 mg)	86 (100%)
Clopidogrel (75 mg daily)	67 (77.9%)
Prasugrel (10 mg daily)	19 (22.1%)
ACE inhibitors	66 (76.7%)
Angiotensin receptor blockers	21 (24.4%)
Beta blockers	75 (87.2%)
Proton pump inhibitors	75 (87.2%)
Calcium-channel blockers	16 (18.6%)

Abbreviations: ACE inhibitors, angiotensin converting enzyme inhibitors*; BMI,* body mass index*; CAD,* coronary artery disease.

Laboratory methods. Blood samples were obtained by clean venipuncture from an antecubital vein using a 21-gauge butterfly needle (0.8 × 19 mm; Greiner Bio-One, Kremsmünster, Austria) after overnight fasting. To avoid procedural deviations, all blood samples were collected 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, blood was drawn into a 3.8% sodium citrate Vacuette tube (Greiner Bio-One; 9 parts of whole blood, 1 part of sodium citrate