Platelet reactivity and cardiovascular events

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GENERAL INTRODUCTION

CARDIOVASCULAR DISEASE AND PLATELETS

In the previous century, cardiovascular diseases have emerged as the predominant cause of death and disability in Western countries.[1] By 1950, about one out of every three men developed cardiovascular disease before the age of 60. In the last decades, cardiovascular mortality has dramatically decreased due to major steps forward in prevention and treatment of cardiovascular disease. In the Netherlands, the incidence of cardiovascular mortality reduced by approximately 40% in men and 20% in women between 1980 and 2008.[2] The incidence of cardiovascular mortality nearly halved in both men and women when changes in age distribution are taken into account. Nevertheless, in 2008, still 30% of the total mortality was attributable to cardiovascular diseases, which is comparable with the mortality due to cancer. Furthermore, in 2008, 345,830 persons were hospitalized due to cardiovascular disease, which is nearly twice as many as in 2000.[2] Therefore, cardiovascular disease remains a major cause of morbidity and mortality in the 21st century and research into the etiology, prediction and prevention of cardiovascular disease remains of paramount importance.

Ischemic cardiovascular disease, particularly coronary artery disease and ischemic stroke, accounts for up to half the mortality and morbidity associated with cardiovascular disease.[2] Ischemic cardiovascular disease is a multicausal disease with smoking, dyslipidemia, hypertension, diabetes, and obesity as major risk factors.[1,3,4] Development of atherosclerosis with plaques vulnerable to rupture or erosion is fundamental in the pathophysiology of arterial thrombosis. Platelets play a key role in atherothrombosis, both in the development of atherosclerosis and in triggering the acute onset of thrombosis.[5-7]

PLATELET PHYSIOLOGY AND ATHEROTHROMBOSIS

Blood platelets or thrombocytes are small (2 to 3 μm in diameter), anucleate cellular fragments derived from megakaryocytes that play an essential role in maintaining normal hemostasis. The normal platelet count is 1.5 to 4.5 · 10^8/mL and platelets have an average life span of 5 to 9 days. Platelets have first been described by Max Schultze in 1865, and in 1882 Giulio Bizzozero for the first time showed their involvement in coagulation.[8] Since then, enormous progress has been made in understanding of involvement of platelets in normal hemostasis and atherothrombosis. Upon vessel wall damage such as the rupture of an atherosclerotic plaque, platelets adhere to the extracellular matrix through several receptors, of which the glycoprotein Ib/V/IX receptor complex with von Willebrand Factor as major ligand and the collagen receptors glycoprotein Ia and VI are most important.[5] As schematically illustrated in Figure 1, following adhesion to the damaged vessel wall, platelets become activated. This is amplified by several agonists including adenosine diphosphate (ADP), thromboxane A_2, thrombin and epinephrine. Additional platelets are recruited and become also activated and finally, the glycoprotein IIb/IIIa receptor is activated and platelets aggregate with each other to form a hemostatic plug.[5]

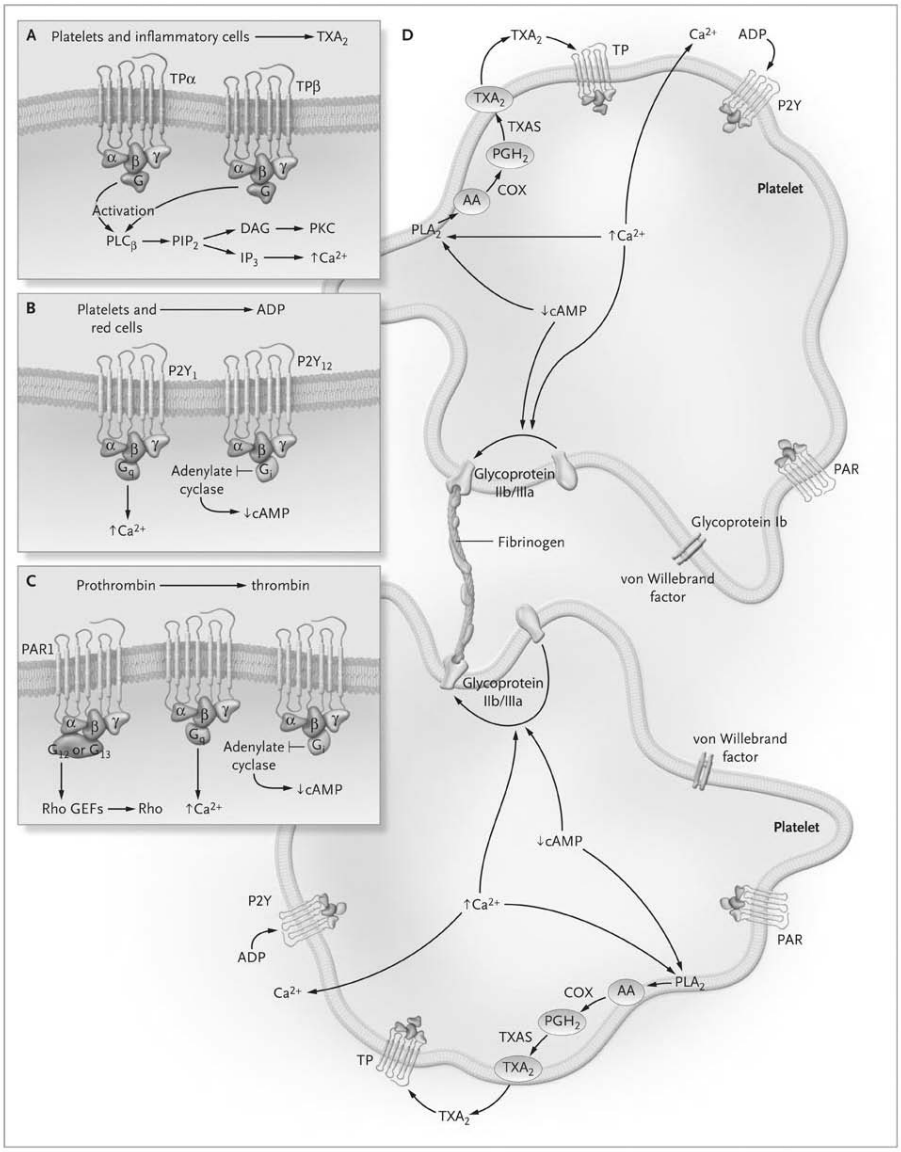

Figure 1 – Agonists, receptors, and effector systems in platelet activation. The activation of platelets is induced by the interaction of several agonists with receptors expressed on the platelet membrane. Panels A, B, and C depict outside-in signaling mediated by thromboxane A_2 (TxA_2), adenosine diphosphate (ADP), and thrombin, respectively. Panel D depicts inside-out signaling. The effects of agonists mediated by the decrease in cyclic AMP (cAMP) levels and increase in intracellular calcium (Ca^{2+}) levels lead to platelet aggregation through the change in the ligand-binding properties of the glycoprotein IIb/IIIa, which acquires the ability to bind soluble adhesive proteins such as fibrinogen and von Willebrand factor. Decreased cAMP levels and increased Ca^{2+} levels also lead to release of ADP and TxA_2 again, which induces further platelet activation and aggregation. NEJM 2007;357:2485. Used with permission.

Next to adhesion and aggregation in the acute response to a disrupted atherosclerotic plaque, platelets could also contribute to development of atherosclerosis.[5-7] Atherosclerosis is a chronic inflammatory disease and various inflammatory stimuli could affect rolling and subsequent adhesion of platelets along activated intact endothelium leading to platelet activation. Activated platelets release large quantities of growth factors, cytokines, chemokines and interleukins from their granules, which have multiple proinflammatory effects (Figure 2). These factors induce platelet-endothelium interactions, platelet-leukocyte-interactions, recruitment of leukocytes to the site of inflammation, and differentiation of monocytes into macrophages initiating the process of plaque formation and atherosclerosis.[9,10] To summarize, platelets exert both acute and chronic effects in the development of atherothrombosis.

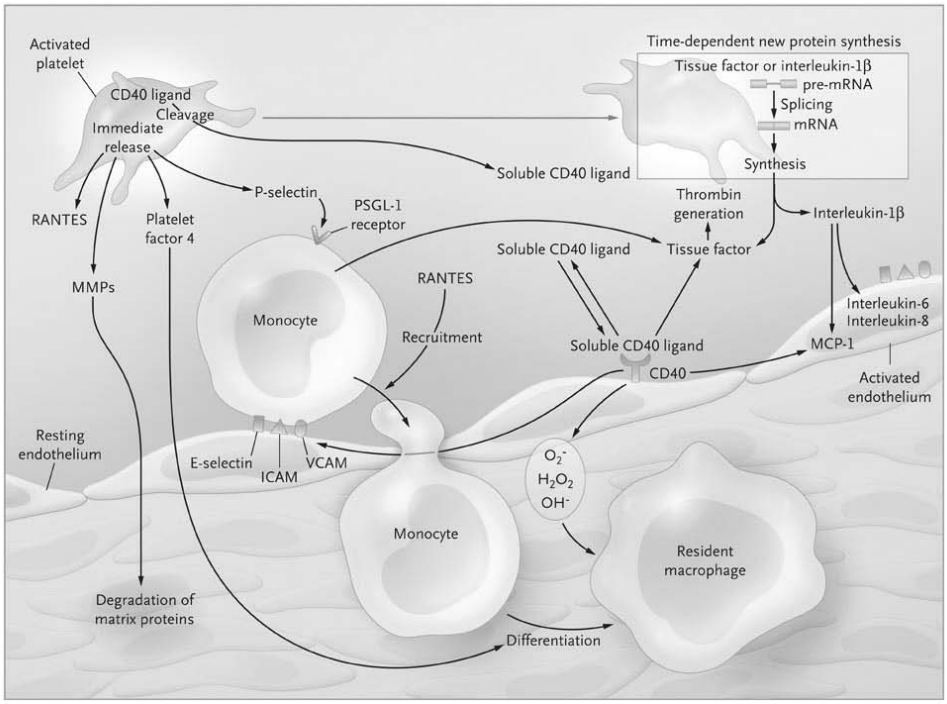

Figure 2 – Platelet-derived mediators of the inflammatory response. Activated platelets release inflammatory and mitogenic substances into the microenvironment, primarily altering the chemotactic, adhesive, and proteolytic properties of the endothelium. Activated platelets also release chemokines that trigger the recruitment of monocytes or promote the differentiation of monocytes into macrophages, as well as matrix-degrading enzymes. NEJM 2007;357:2486. Used with permission. Copyright © 2007 Massachusetts Medical Society. All rights reserved.

PLATELET PHARMACOLOGY

Clinically, the involvement of platelets in atherothrombosis has been firmly established by large trials showing that antiplatelet therapy reduces the risk of cardiovascular events. Platelets can be inhibited through various pharmacological targets, reflecting the different pathways of platelet activation (Figure 1). Aspirin (acetylsalicylic acid) is the most thoroughly evaluated antiplatelet drug and is a cornerstone in cardiovascular prevention. The effect of low-dose aspirin is most likely based on the permanent inactivation of cyclooxygenase-1 (COX-1) through blockade of the COX-channel by the acetylation of serine residue 529, which results in an irreversible inhibition of the production of thromboxane A_2 from arachidonic acid by platelets.[11] In subjects with manifest cardiovascular disease, aspirin decreases the risk of recurrent events with approximately 25%.[12] In a primary prevention setting, the beneficial effect of aspirin is largely offset by an increased risk of bleeding in most patients.[13]

P_2Y_{12}-receptor blockers are used after acute coronary syndrome or percutaneous coronary intervention. Ticlopidine, clopidogrel and prasugrel belong to the group of structurally related antiplatelet agents called thienopyridines which are metabolized in the liver to active metabolites and irreversibly block the P_2Y_{12}-receptor. Cangrelor and ticagrelor do not need metabolization to become active and block the P_2Y_{12}-receptor reversibly. The clinical effectiveness of addition of clopidogrel to aspirin therapy to prevent cardiovascular events after PCI has been shown repeatedly.[14-16] The new antiplatelet drugs prasugrel and ticagrelor have been shown to be superior to clopidogrel.[17,18]

Other antiplatelet drugs include the glycoprotein IIb/IIIa inhibitors which antagonize the glycoprotein IIb/IIIa receptor and inhibit the final pathway of platelet aggregation, which are used during coronary interventions,[19] and dipyridamole, which inhibits platelets via various mechanisms and is particularly effective in patients with ischemic stroke.[20]

INTERINDIVIDUAL VARIATION IN PLATELET REACTIVITY

For a long time, physicians have used a "one-size-fits-all" approach when treating patients with antiplatelet drugs. However, in the last two decades the awareness has grown that platelet reactivity may vary among individuals, even when treated with antiplatelet drugs, and that this variation may influence the risk of cardiovascular events. In this context, although widely debated, the terms "aspirin resistance" and "clopidogrel resistance" have been raised.[21-24] Much is unknown about the determinants, the prevalence, the clinical consequences and the treatment of these phenomena.

The term antiplatelet drug resistance, particularly aspirin resistance, was originally used to describe the situation in which cardiovascular events occurred despite use of antiplatelet therapy. However, as atherothrombosis is a multifactorial process in which many more factors than platelet reactivity play a role, it is not surprising that antiplatelet

therapy does not prevent all events, even if sufficiently reaching its pharmacological target. This phenomenon is therefore better described as "treatment failure" rather than antiplatelet drug resistance.[25] Later, laboratory definitions of antiplatelet drug resistance emerged. Aspirin and clopidogrel resistance were defined as high *ex vivo* platelet reactivity despite the use of aspirin or clopidogrel using a laboratory test. This definition has been further refined based on the used test, because platelet reactivity can be tested by many methods, varying from highly specific for an antiplatelet drug to a more global test. The perception has grown that the term "resistance" should be limited to situations in which a drug does not hit its pharmacological target, i.e. inhibition of thromboxane A_2 production by COX-1 in case of aspirin and inhibition of the P_2Y_{12}-receptor in case of clopidogrel, as documented by a highly specific test.[26] When less specific tests are used, the test will only partly reflect the thromboxane A_2 or P_2Y_{12} pathway and high platelet reactivity does not automatically imply that the drug does not hit its pharmacological target. Therefore, when more global tests are used it is better to use the term "persistent platelet reactivity despite treatment" or "high on-treatment residual platelet reactivity" rather than antiplatelet drug resistance.[26] Another definition is "antiplatelet drug responsiveness", indicating the absolute difference in platelet reactivity before and after treatment, although this term has also frequently been used as a synonym for on-treatment platelet reactivity. Finally, when platelet reactivity is tested in antiplatelet drug-naïve subjects, the term "basal platelet reactivity" has been used.

The different chapters in this thesis reflect the evolution of definitions of platelet reactivity. In all chapters except chapter 7, which concerns "basal platelet reactivity", we studied what is currently described as "high on-treatment platelet reactivity", although the terms "persistent platelet reactivity despite use of aspirin" (chapter 2), "laboratory aspirin resistance" (chapter 3), "clopidogrel non-responsiveness" (chapter 4) were also used to describe the same phenomenon in some chapters. We used both specific and non-specific tests to the effect of aspirin and clopidogrel to quantify on-treatment platelet reactivity, as described in the different chapters.

AIMS AND OUTLINE OF THIS THESIS

This thesis addresses variation in platelet reactivity in relation to occurrence of cardiovascular events. The aim of this thesis is to provide insight into the extent, the causes and the clinical consequences of interindividual variation in platelet reactivity.

In **chapter 2** we systematically review and quantify what is known from previous research about the prevalence of high on-aspirin platelet reactivity. We also examine the effect of several factors influencing high platelet reactivity. **Chapter 3** addresses the relation between high on-aspirin platelet reactivity and recurrence of cardiovascular events in subjects with established cardiovascular disease using aspirin. To this end we again systematically reviewed all available studies that address this question. In **chapter 4** data analogous to those of chapters 2 and 3 are presented for high on-clopidogrel platelet reactivity in subjects undergoing percutaneous coronary intervention.

A limitation of the results from Chapters 2 to 4 is that numerous different tests were used to quantify high on-treatment platelet reactivity in the studies included in the analyses. The data were too scarce to draw conclusions about individual tests of platelet reactivity in relation to clinical endpoints. Therefore, we designed a study to perform a head-to-head comparison of a variety of tests of platelet reactivity in subjects with established cardiovascular disease using aspirin. We included both tests specific for the effect of aspirin and more global tests. The results of this study are presented in Chapter 5 and Chapter 6. In **chapter 5** we examine the correlation between the different tests of platelet reactivity as well as the determinants of the different tests. **Chapter 6** addresses the relation between high on-aspirin platelet reactivity according to the different tests and recurrent cardiovascular events.

As platelets also contribute to inflammation and the development of atherosclerosis, we hypothesized that interindividual variation in basal platelet reactivity also influences the risk of a first atherothrombotic event in healthy individuals. To test this hypothesis, in **chapter 7** we report on a study into the relation between high basal platelet reactivity as measured by several circulating markers that are secreted into the circulation by platelets upon activation, and myocardial infarction in young women.

One of the most important determinants of variation in platelet reactivity could be genetic variation, which would have a life-long effect on platelet reactivity. In **chapter 8**, we examine the relation of polymorphic variation in the gene encoding platelet glycoprotein VI, a platelet collagen receptor, with platelet activation and aggregation, as well as the risk of first myocardial infarction, recurrent cardiovascular events and mortality.

One of the most important risk factors for cardiovascular disease is arterial hypertension. Aspirin is usually thought to have no effect on blood pressure. However, some recent studies indicate that aspirin may influence blood pressure, depending on the time of intake.[27,28] In those studies, use of aspirin decreased blood pressure when taken at bedtime, whereas this effect was not observed when aspirin was taken on awakening. However, a biologically plausible mechanism for this phenomenon has not been revealed. In **chapter 9** we present a randomized controlled trial in which we compare aspirin intake on awakening with intake at bedtime in relation to various determinants of blood pressure to examine the underlying mechanism of this potential time-dependent effect of aspirin on blood pressure.

In **chapter 10** we summarize and discuss the results of the different studies presented in this thesis.

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