Thrombosis Research (2006) **118**, 371–380

THROMBOSIS RESEARCH

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REGULAR ARTICLE

GPVI-deficient mice lack collagen responses and are protected against experimentally induced pulmonary thromboembolism

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Received 22 April 2005; received in revised form 14 July 2005; accepted 2 August 2005
Available online 2 September 2005

KEYWORDS
Platelet;
GPVI-knockout;
Aggregation;
Adhesion;
Bleeding time

Abstract Platelet glycoprotein VI (GPVI) is now considered to be a major player in platelet–collagen adhesive interactions leading to thrombus formation. GPVI blockade, or its depletion, has been shown in mice to result in complete protection against arterial thrombosis, without significant prolongation of bleeding time. GPVI may therefore represent a useful antithrombotic target. In order to reaffirm the role of GPVI in platelet–collagen interactions, we developed GPVI^{null} mice by targeted disruption methodology. GPVI^{null} mice platelets failed to respond to a high dose of fibrillar collagen, or convulxin, a GPVI agonist, but showed a normal response to other agonists such as ADP, PMA and arachidonic acid. We report, for the first time, that a proportion of GPVI^{null} mice is protected against lethal thromboembolism, induced by the infusion of a mixture of collagen and epinephrine. Greater than 55% of GPVI^{null} mice survived the challenge, whereas the maximal survival from the other genotypes was 17% ($n=18$ per genotype). Washed platelets obtained from GPVI^{null} mice showed >90% reduction in adhesion to fibrillar collagen under static conditions. Platelet adhesion to collagen under dynamic conditions using a high shear rate (2600 s^{-1}) was dramatically reduced using blood from GPVI^{null} mice, while platelets from wild-type and heterozygous animals showed a similar amount of adhesion. Animals from each genotype had essentially similar tail bleeding time, suggesting that a

Abbreviations: AA, arachidonic acid; PMA, phorbol 12-myristate 13-acetate; CVX, convulxin; PGE_1, Prostaglandin1; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene glycol-bis-(2 aminoethylether) *N*,*N*′,*N*′-tetraacetic acid; BSA, bovine serum albumin; PWB, platelet wash buffer; PRP, platelet-rich plasma; PPP, platelet-poor plasma; PCR, polymerase chain reaction.

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0049-3848/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2005.08.001

complete deficiency of GPVI, at least in mice, does not result in an enhanced bleeding tendency. These observations clearly establish that blockade of GPVI may attenuate platelet–collagen interactions without adversely affecting the bleeding time.
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Introduction

Thrombosis is initiated by the interaction of platelets with components of the blood vessel wall, exposed either by injury, or by a pathological process such as atherosclerosis [1]. When the endothelium is damaged, platelets rapidly interact with components of the exposed subendothelium, initiating a series of reactions leading to the formation of a stable platelet plug and cessation of any blood loss. However, when a pathological process, such as the rupture of an atherosclerotic plaque, exposes the subendothelium, the consequences of thrombosis may be undesirable. Collagen is considered to be the most thrombogenic component of the subendothelium, interacting with several platelet surface receptors [2]. In a high shear environment, such as an artery, platelets initially bind indirectly to collagen via von Willebrand factor (vWF), which coats the exposed collagen and acts as a bridge between the subendothelium and the platelet surface glycoprotein (GP) Ib–IX–V complex. This interaction serves to tether the platelet to the vessel wall, but the affinity of this interaction is not sufficient to arrest the downstream motion of the platelet. Activation of integrin receptors $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$ allows binding with collagen and vWF interaction between platelets and vessel wall acts as an anchor to firmly attach the platelet [3,4]. Recent investigations of the role of glycoprotein VI suggest that the firm adhesion of platelets is achieved through the participation of two major collagen receptors, namely GPVI and $\alpha_2\beta_1$, described as "two step two-site model" by Neiswandt and Watson [5]. The initial contact with the extra cellular matrix is mediated predominantly through GPIb–V–IX–vWF and GPVI–collagen interaction. In the second step, GPVI–collagen interactions induce conversion of integrins to a high-affinity state and release of the secondary agonists ADP and thromboxane A_2 (TXA2). Finally, firm adhesion of platelets to collagen through activated $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$ via vWF results in sustained GPVI signaling, enhanced release, and procoagulant activity. Released ADP and TXA2 amplify integrin activation of adhered platelets and mediate thrombus growth by activating additional platelets. Expressed only in platelets and megakaryocytes, GPVI is a member of the immunoglobulin superfamily of receptors and is found as a non-covalent heterodimeric complex with the γ-chain of the Fc receptor [6]. Concomitant expression of FcRγ-chain seems to be required for the surface expression of GPVI since FcRγ-chain-null mice are deficient in GPVI [7]. The co-expression of GPVI with FcRγ-chain is essential since collagen binding to GPVI results in platelet signaling via the immunoreceptor tyrosine-based activation motif (ITAM) present in the FcRγ-chain [5,6,8,9].

Although the involvement of GPVI in platelet–collagen interactions has been recognized for many years, it has recently been suggested to be the central receptor for collagen–platelet interactions [5]. Platelets from several GPVI-deficient patients failed to respond to collagen. The reduced amounts of GPVI, or its lack may have resulted from autoantibody-induced shedding of the extracellular domain, or possible gene deletion [10–15]. Mice made GPVI-deficient by the administration of an anti-GPVI antibody also exhibited similar impairment of collagen-induced platelet aggregation [16]. In addition to lacking collagen-induced platelet aggregation, these animals showed protection against experimentally induced pulmonary thromboembolism [16]. Similarly, in a recently published report of a GPVI-knockout mouse, collagen-stimulated functions such as platelet aggregation and ex vivo thrombus formation on type I collagen was abolished [17]. Although GPVI-deficient patients lacked platelet–collagen interactions, only a mild bleeding tendency was noted. Similarly, lack of platelet response to collagen stimulation and a slightly prolonged bleeding time was reported in GPVI-depleted mice [16], but the majority of GPVI-knockout mice did not show any increase in tail bleeding time [17]. These observations clearly suggest that the absence of GPVI has no major impact on bleeding and therefore blockade of GPVI may be expected to attenuate platelet–collagen interactions without adversely affecting hemostatic functions, such as bleeding time. GPVI might therefore be an attractive therapeutic antithrombotic target lacking some of the drawbacks of current compounds targeting platelet integrins.

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