

Soluble Glycoprotein VI (GPVI) in Human Plasma: Shedding of GPVI from Platelets Induced by Coagulation

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Aim

The aim of this study is to evaluate the effect of coagulation on the surface expression of the platelet collagen receptor, glycoprotein VI (GPVI). We have previously shown that collagen, collagen-related peptide (CRP), snake toxins or anti-GPVI antibodies induce metalloproteinase-mediated ectodomain shedding of GPVI, generating an ~55-kDa soluble fragment (sGPVI), and an ~10-kDa remnant fragment that remains platelet-associated.

Methods

We used a newly-developed enzyme-linked immunosorbent assay (ELISA) to measure sGPVI levels in human plasma from healthy individuals, normal platelet-rich plasma where coagulation was experimentally induced, and plasma from patients with disseminated intravascular coagulation (DIC).

Results

Initial studies showed that plasma sGPVI levels in 192 healthy individuals were 19.5 ± 15.4 (2x s.d.) ng/mL, and levels were independent of age, gender or common GPVI polymorphisms (associated with Gln317/Leu substitution). However, sGPVI levels were markedly elevated following coagulation: First, while plasma sGPVI was independent of the anticoagulant used for blood collection (acetate-citrate-dextrose (ACD), citrate, or EDTA), collecting blood into a silica-coated coagulation tube and analysing serum from clotted blood showed markedly elevated sGPVI levels (124 ng/mL *cf.* 29 ng/mL in ACD-anticoagulated plasma). Second, inducing coagulation in normal citrated platelet-rich plasma by recalcification with or without added tissue factor resulted in increased sGPVI. This increase in sGPVI followed initial thrombin generation and peaked after 30 minutes at ~7-10-fold baseline levels. Shedding was strongly inhibited by hirudin (thrombin inhibitor) or GM6001 (broad spectrum metalloproteinase inhibitor), suggesting activated thrombin induced metalloproteinase-mediated GPVI shedding from platelets. Third, initial analysis of patients with DIC showed elevated plasma sGPVI consistent with increased GPVI shedding *in vivo* associated with coagulopathy.

Conclusions

These findings reveal that coagulation results in ectodomain shedding of platelet GPVI, and significant elevation of sGPVI levels in plasma. GPVI depletion limiting adhesion-dependent platelet activation may compensate for increased procoagulant activity in disease states.

No conflicts of interest to disclose