Protease activated receptors and glycoprotein VI cooperatively drive the platelet component in thromboelastography

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Background
Platelet activation signaling

Methods

Results

Figure 1: Role of platelets and platelet contraction in TEG

Figure 2: Role of uPAR/integrin activation and ligand binding in TEG

Figure 3: Role of Rap1 GTPase signaling in TEG

Figure 4: Role of platelet PAR4 and GPVI in TEG

Figure 5: TEG clotting parameters were assessed in citrated whole blood samples via recombinant platelet agonists (WT) or WT mice + deplete platelets (WT + depleted) via injection of anti-GpIIb antibody. To test platelet-mediated contraction, WT samples were incubated with GM6001 or genipin (0.1% mg/ml) for 10 minutes prior turning TEG assay. Statistical significance was determined using either unpaired Student’s t-test or one-way ANOVA. Symbols directly over bars represent significant results compared to WT control **P<0.01, ***P<0.001.

Figure 6: Role of PAR1/PAR4 and Syk in human blood TEG

Results (continued)

Figure 5: Citrated whole blood samples were analyzed from WT mice or mKO mice treated with a mKO-specific monoclonal antibody (JAQ1) had no reductions in R, and MA but no change in R time. Statistical significance was determined by one-way ANOVA with Tukey’s multiple comparison test. Symbols directly over bars represent significant results compared to WT control **P<0.01, ***P<0.001.

Figure 6: Volume platelet samples were analyzed with the addition of DDQ, vonopram (Vita), BMS-986123, and PRI-7627 to inhibit PAR1, PAR4, and Syk, respectively. Statistical significance was determined by one-way ANOVA with Tukey’s multiple comparison test. Symbols directly over bars represent significant results compared to control **P<0.01, ***P<0.001.

Summary

- Platelet depletion and glycoprotein IIb/IIIa antibodies showed a significant decrease in R and MA but no change in R time.
- Significant reductions in R and MA were seen in WT blood treated with blocking antibody to uPAR (20 µg/mL) and in Thrombin (20 nM).
- Using both isoforms of Rap1 (slap1 and αIIbβ3) had significant reductions in R and MA. But were also thrombogenic/cytotoxic.
- While reduced levels of the specific agonists of the two major pathways for Rap1 activation (CTAP1 and αβ3) appeared to have no impact on parameters.
- PAR4-αIIbβ3 mKO mice treated with anti-GP IIb/IIIa antibody showed a similar reduction in R in an unstimulated platelet setting, while intracellular adhesion of the frontline or of the two major pathways for Rap1 activation (CTAP1) and αβ3 appeared to have no impact on parameters.

Conclusions

- In trauma-associated hemorrhage, a defect in the MA would typically call for platelet transfusions.
- According to our findings, substantial platelet activation (platelet release and degranulation) may not always be associated with significant platelet contribution to MA.
- A patient with a normal MA in standard TEG may have platelet defects (i.e., thrombocytopenia or platelet dysfunction) and not require platelet transfusions.
- It is possible to use complementary measurements in a panel to identify patients with platelet dysfunction.
- Platelet transfusion for patients with normal MA should be cautiously considered.

Future Directions

- It would be of great interest to further investigate the ability of TEG to assess the hemorrhagic efficacy of platelet transfusions for patients with abnormal platelet dysfunction.

Acknowledgments


This study was funded by the National Institutes of Health, American Society of Hematology, and Bloodwise Foundation (now Association for the Advancement of Blood and Blood-related Foundation).