Measurement of in situ plasmin generation in murine plasma reveals dependency on fibrin formation and plasminogen-fibrin interactions

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**Background.** Thrombin generation (TG), fibrin formation, and clot dissolution (plasmin-mediated fibrinolysis) are tightly-orchestrated processes essential for hemostasis. Disruption of the delicate balance between these processes contributes to bleeding and thrombotic disorders. A dearth of in vitro assays sensitive to in situ plasmin generation (PG) in plasma has limited understanding of fibrinolytic mechanisms and the molecular role of fibrin dynamics during fibrinolysis.

**Aim.** Develop and characterize a plasma PG assay to determine the role of fibrinolytic system components on the kinetics of in situ PG.

**Methods.** TG and PG were measured with fluorogenic substrates, and fibrin formation by turbidity, during clot formation in platelet-poor plasmas from wildtype and genetically-modified mice.

**Results.** PG can be characterized by kinetic parameters, including: lag time, velocity, peak, and endogenous plasmin potential. Plasma dilution decreased the plasmin peak and the endogenous plasmin potential. PG depended on tissue plasminogen activator (tPA) concentration and was predictably absent in plasminogen-deficient plasma. Experiments with plasmas from wildtype, \textit{Fga}\textsuperscript{-/-}, and \textit{F13a1}\textsuperscript{-/-} mice, and mice expressing mutant fibrinogen that cannot polymerize (\textit{Fgn}\textsuperscript{AEK}), showed PG was mediated by the rate of fibrin formation and total fibrin formed, but not by fibrin crosslinking. Experiments with fibrin polymerization and fibrinolysis inhibitors demonstrated biphasic PG, suggesting plasminogen binding to fibrin and fibrin degradation products modulates complex PG kinetics.

**Conclusion.** This novel PG assay requires only small sample volumes and is sensitive to tPA, plasminogen, fibrin(ogen), and fibrin formation kinetics. This assay may be used to evaluate the contribution of fibrinolysis to hemostatic and thrombotic disease.

I would like to apply for the Outstanding Abstract Award

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