Prothrombinase Assembled with Factor V Leiden is Resistant to Inhibition by Tissue Factor Pathway Inhibitor α Lowering the Procoagulant Threshold for Initiation of Coagulation

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Factor V (FV) assembles with factor Xa (FXa) into prothrombinase, the enzymatic complex that converts prothrombin to thrombin. Tissue factor pathway inhibitor α (TFPIα) inhibits prothrombinase by high affinity interactions with FXa-activated FV and the FXa active site, thereby blocking the initiation of coagulation. FV Leiden (FVL) is strongly linked to venous thrombosis through its resistance to degradation by activated protein C (aPC), which enhances the propagation of coagulation. FVL combined with a 50% reduction in TFPI causes severe thrombosis and perinatal lethality in mice, suggesting that FVL also promotes the initiation of coagulation. To examine this possibility, thrombin generation assays initiated with limiting FXa were performed with control or FVL plasma and platelet-rich plasma (PRP). The activation threshold for thrombin generation was 10 to 20 pM FXa in 10 control plasmas, but was 5 pM in 4 of 10 homozygous FVL plasmas. FVL PRP had a similar decrease in the activation threshold. The differences in activation threshold were totally normalized by an anti-TFPI antibody, while exogenous TFPIα and a FV-binding peptide that mimics TFPIα had reduced anticoagulant activity in FVL plasma, revealing that the procoagulant effects of FVL in these assays rely on TFPIα. Next, FVL plasmas were studied in fibrin clot formation assays, as they are sensitive to small amounts of thrombin. In reactions activated with 0.5 pM FXa, 1 of 8 control plasmas, compared to 7 of 8 homozygous FVL plasmas, clotted within 60 minutes, with differences again normalized by the anti-TFPI antibody. In prothrombinase activity assays using purified proteins, TFPIα was a 1.7-fold weaker inhibitor of prothrombinase assembled with FVL compared to FV. Thus, in addition to its aPC-mediated effect on the propagation of coagulation, FVL is resistant to TFPIα inhibition, exerting a procoagulant effect on coagulation initiation. This is evident in responses to small stimuli, where TFPIα blocks clotting in plasmas with FV but not FVL. The TFPIα-mediated modulation of the procoagulant threshold may explain the severe perinatal thrombosis in FVL mice with decreased TFPI and be clinically relevant in the clotting associated with oral contraceptives, which cause acquired TFPI deficiency.

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