Evaluation of Hemostatic Effect of BAY 86-6150, a Recombinant FVIIa Variant in Antibody-Induced Hemophilic Whole Blood Under Flow Conditions

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Introduction

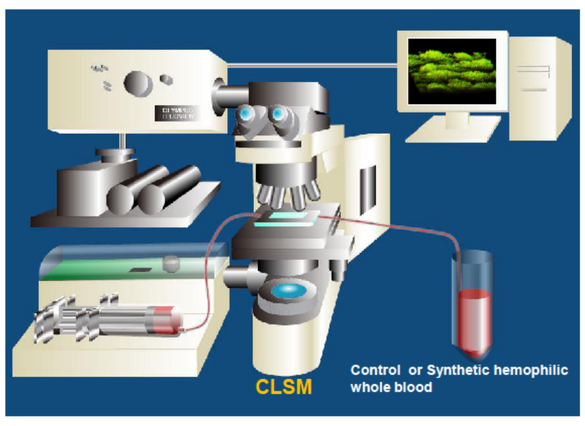
BAY 86-6150, an activated recombinant factor VII (rFVIIa) variant, is currently in clinical development as a therapeutic agent for people with hemophilia (PWH) with inhibitors. Our previous studies have shown that BAY 86-6150 exhibits enhanced activated factor X (FXa) generation on the surface of activated platelets *in vitro* and increased circulation time resulting in prolonged efficacy *in vivo*.

Aim

To further investigate the antihemophilic properties of BAY 86-6150, we evaluated the effect of BAY 86-6150 on thrombus formation under whole blood flow conditions with a high shear rate (1500 s^{-1}) using an *in vitro* perfusion chamber system.

Methods

Whole blood was perfused over a collagen-coated glass plate in a parallel-plate flow chamber, and the thrombus formation process on the collagen surface was monitored by confocal laser scanning microscopy. The extent of intra-thrombus fibrin generation, detected by fluorescently-labeled anti-fibrin specific monoclonal antibody, was evaluated as a ratio of intensity of the Cy3-fluorescence (orange:fibrin) relative to that of FITC-fluorescence (green:fibrinogen).The ability of BAY 86-6150 to promote clot formation in whole blood from healthy donors rendered hemophilic by anti-factor VIII antibody (final inhibitor titer: 5 Bethesda U/ml) was investigated (Fig 1).



In evaluation of factor VIIa deposition within thrombi, a mouse anti-FVII antibody was used instead of the anti-fibrin antibody (Fig 4B).

Flow chamber system to analyze fibrin generation within platelet thrombi under whole blood flow conditions

Results

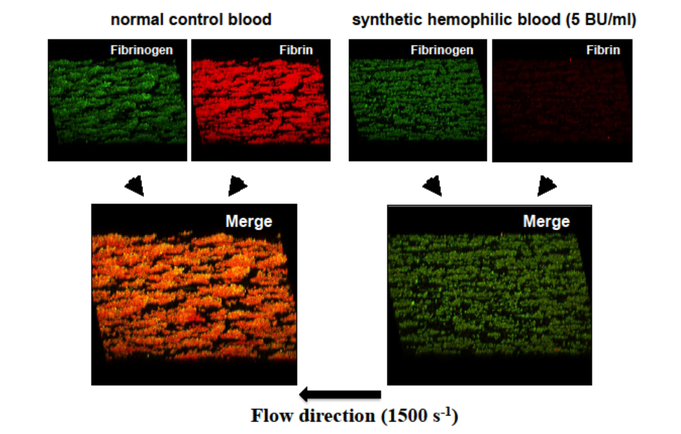


Fig 1: 3-D images of fibrin generation in normal control or synthetic hemophilic thrombi generated on collagen surface under high shear rate

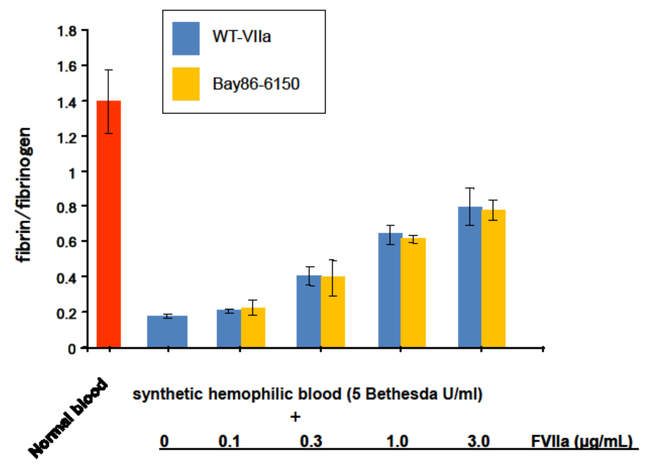


Fig 3: Both BAY 86-6150 and WT-FVIIa increase fibrin deposition in a dose-dependent manner on a collagen-surface under a high shear rate condition.

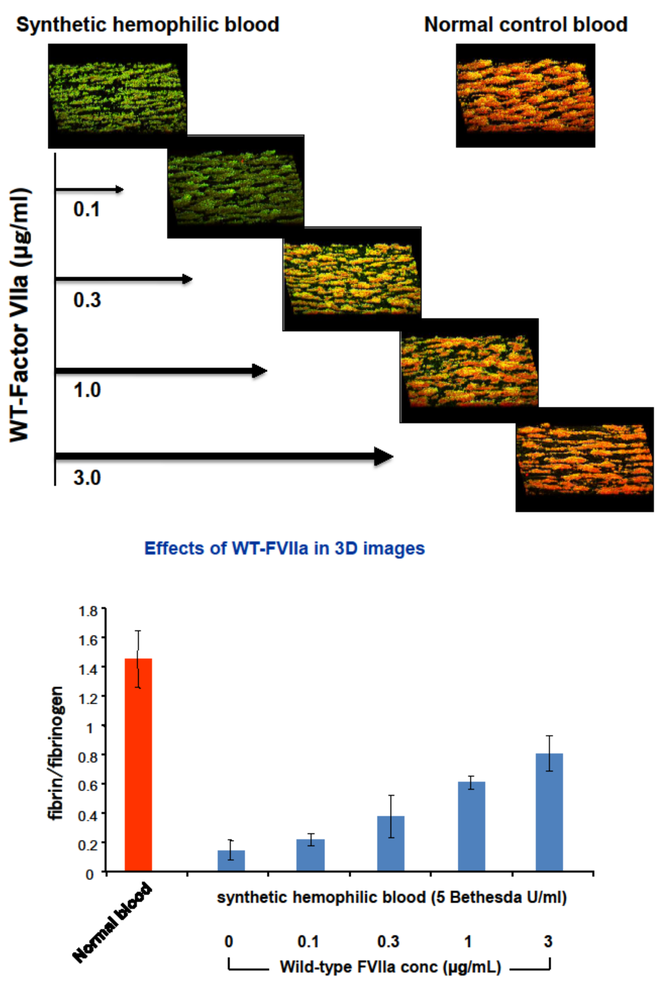


Fig 2: WT-FVIIa significantly improved the impaired fibrin generation within hemophilic thrombi in a dose-dependent manner under whole blood flow condition.

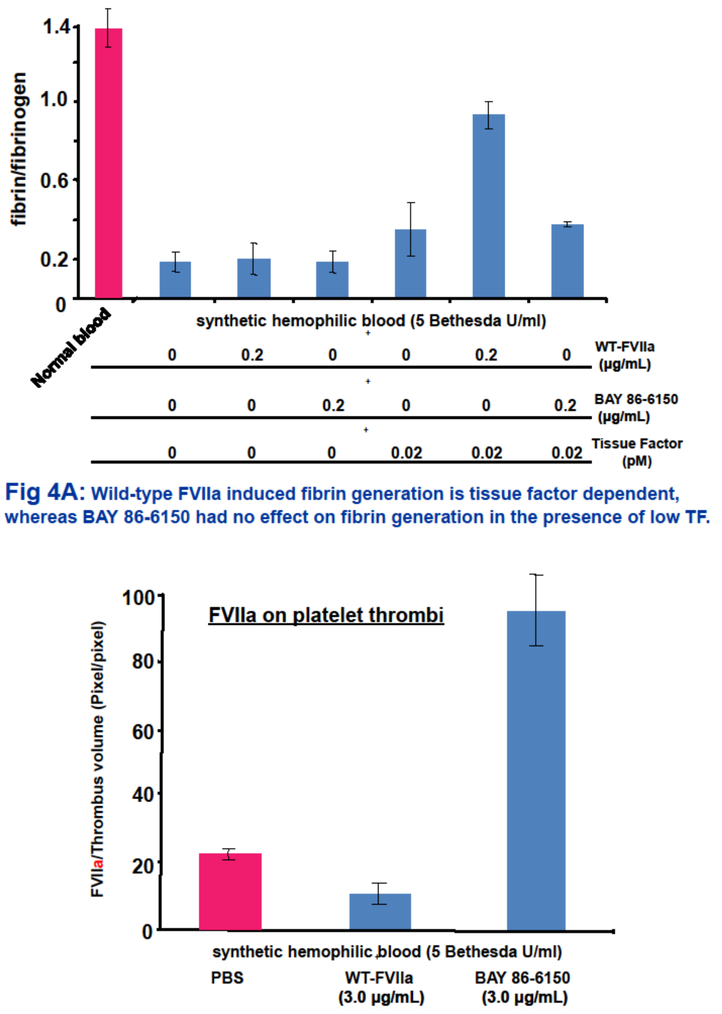


Fig 4A: Wild-type FVIIa induced fibrin generation is tissue factor dependent, whereas BAY 86-6150 had no effect on fibrin generation in the presence of low TF.

Fig 4B: Significant higher FVIIa deposition in intra-platelet thrombi was observed in synthetic hemophilic blood in the presence of BAY 86-6150, as compared to WT-FVIIa.

Summary:

1.Both BAY 86-6150 and wild-type rFVIIa increased the fibrin generation within hemophilic thrombi in a dose-dependent manner, nearly normalizing at concentrations > 0.3 mg/ml (~6 nM) (Fig 2 and Fig 3).

2.The fibrin generation and platelet thrombi induced by BAY 86-6150 in antibody-induced hemophilic blood are independent of tissue factor under flow conditions (Fig 4A).

3.Immunostaining of platelet thrombi with anti-FVII antibody detected a 5- to 10-fold higher amount of FVIIa in thrombi generated in the presence of BAY 86-6150 relative to wild-type FVIIa under flow conditions (Fig 4B). This is consistent with the higher affinity of BAY 86-6150 for activated platelets.

Conclusion

Our results demonstrated that BAY 86-6150 is a unique and TF-independent FVIIa variant with enhanced efficacy, particularly at sites of vascular injury where hemostatic platelet thrombi are formed.

Disclosures

 # Jian-Ming Gu, Ji-Yun Kim, Derek Sim, Volker Laux, John E Murphy, and Timothy Myles are employees of Bayer HealthCare LLC.

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