Plasma Tissue Factor Pathway Inhibitor (TFPI) Levels in Healthy Subjects and Patients With Hemophilia A and B

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INTRODUCTION

- Hemophilia A, caused by a deficiency of the coagulation factor VIII (FVIII), can result in spontaneous bleeding into joints, muscles, and internal organs.
- About 30% of patients treated with recombinant FVIII (rFVIII) develop anti-FVIII inhibitors and rely on bypassing agents such as recombinant activated factor VII (rFVIIa) and activated prothrombin complex concentrate. Other bypass agents such as anti-tissue factor pathway inhibitor (TFPI) monoclonal antibody (mAb) and the bispecific antibody ACE910 have been used in clinical trials.
- TFPI is a potent inhibitor of factor Xa (FXa) and the FVIIa tissue factor complex in the extrinsic pathway. BAY 1093884 is a fully human mAb against TFPI. BAY 1093884 was developed as a potential bypass agent for patients with hemophilia with or without inhibitors. It restores an insufficient thrombin burst, leading to stable clot formation in hemophilic conditions in vitro, and effectively stops bleeding in vivo.
- The mean plasma TFPI concentration in healthy individuals is ~70 ng/mL (1.6 nM), and about 80% of the circulating TFPI is bound to lipoprotein.[1,2] Some reports indicate that patients with hemophilia B have lower free TFPI levels than those with hemophilia A, irrespective of phenotypic severity.[3]

OBJECTIVE

- The objective of this study was to determine the plasma TFPI concentration in healthy donors and patients with hemophilia using a newly developed functional TFPI capture assay and to evaluate this assay with inhibition of TFPI by anti-TFPI neutralizing antibody (BAY 1093884) in vitro.

METHODS

Study Samples

- The individual plasma samples used for the analyses were as follows:
  - Individual and pooled human plasma anticoagulated with 3.2% sodium citrate (BioreclamationIVT, Westbury, NY) from healthy donors (n=30; 15 men and 15 women, ranging in age from 18–71 years) was used.
  - Plasma samples from 30 patients with severe hemophilia A (n=12), severe hemophilia B (n=9), or severe hemophilia A with inhibitors (n=9) were from HRF Inc (Raleigh, NC). FVIII and FIX activity were confirmed to be <1%.

Free TFPI Enzyme-Linked Immunosorbent Assay (ELISA)

- A quantitative ELISA using FXa as the capture agent was developed and validated to measure TFPI levels in human plasma.
  - Human FXa (from Enzyme Research Laboratory, South Bend, IN) was used to coat the ELISA plate.
  - Recombinant TFPI spiked in TFPI-depleted human plasma (American Diagnostics, Hauppauge, NY) was used for the calibration curve. Plasma samples from healthy individuals and patients were diluted 10-fold with phosphate-buffered saline with 1% bovine serum albumin and used for the analysis.
  - Biotin-labeled anti-human TFPI polyclonal antibody and strepavidin-HRP (R&D Systems, Minneapolis, MN) was used for the detection.

Statistical Analyses

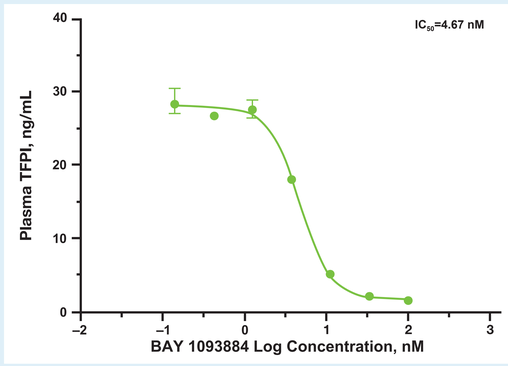
- Statistical significance was assessed using GraphPad Prism 5 (1-way analysis of variance, Dunnett multiple comparison test), and 2-tailed *P* values <0.05 were considered significant.

RESULTS

IC_{50} of Anti-TFPI mAb (BAY 1093884) in Normal Human Pooled Plasma

- The concentration of anti-TFPI antibody (BAY 1093884) that inhibits 50% of TFPI levels (IC_{50}) was determined to be 4.67 nM in normal human plasma using this assay **(Figure 1)**.

Figure 1. IC_{50} of Anti-TFPI Antibody (BAY 1093884) in Inhibition of TFPI Levels in Normal Human Pooled Plasma



IC_{50}=concentration of BAY 1093884 that inhibits 50% of TFPI levels; TFPI=tissue factor pathway inhibitor.

Plasma TFPI Levels in Healthy Individuals and Patients With Severe Hemophilia A and B

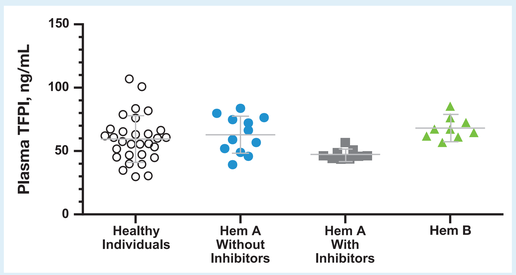
- The plasma TFPI levels (mean ± SD) in healthy individuals and patients with severe hemophilia are shown in **Table 1** and **Figure 2**.

Table 1. Plasma TFPI Levels in Healthy Individuals and Patients With Severe Hemophilia A and B

		Patients		
		Severe Hemophilia A		
	Healthy Individuals (n=30)	Without Inhibitors (n=12)	With Inhibitors (n=9)	Severe Hemophilia B (n=9)
Mean ± SD TFPI, ng/mL	59.5±18.4	62.9±14.6	47.3±4.3	68.1±8.8

TFPI=tissue factor pathway inhibitor.

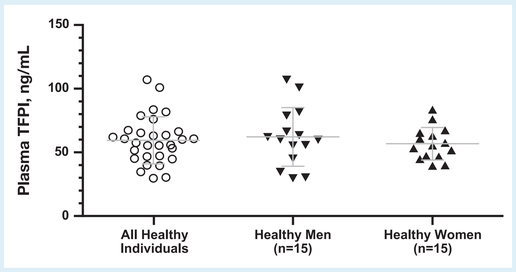
Figure 2. TFPI Levels in Healthy Individuals and Patients With Hemophilia A and B



Horizontal bars represent mean ± SD.
Hem=hemophilia; TFPI=tissue factor pathway inhibitor.

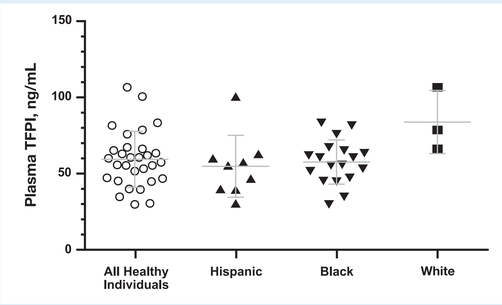
- The plasma TFPI levels in healthy individuals showed
  - No statistical differences with regard to sex or to Hispanic, black, or white race **(Figure 3; Figure 4)**
  - No correlation between age and plasma TFPI levels **(Figure 5)**.

Figure 3. TFPI Levels in Healthy Men and Women



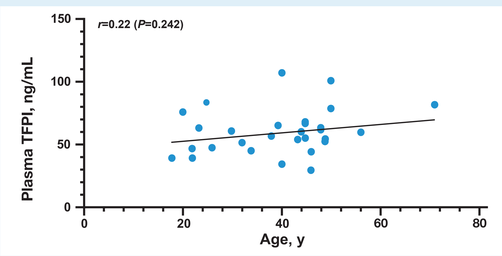
Horizontal bars represent mean ± SD.
TFPI=tissue factor pathway inhibitor.

Figure 4. TFPI Levels in Healthy Hispanic, Black, and White Individuals



Horizontal bars represent mean ± SD.
TFPI=tissue factor pathway inhibitor.

Figure 5. Correlation of TFPI Levels and Age



TFPI=tissue factor pathway inhibitor.

CONCLUSIONS

- The free TFPI ELISA assay shows very good precision, accuracy, and reproducibility and should capture coagulation-relevant forms of TFPI from plasma.
- Plasma TFPI does not appear to be affected by sex or race in healthy individuals or by the deficiency of FVIII or FIX in patients with hemophilia.
- Anti-TFPI antibody (BAY 1093884) can inhibit functional TFPI levels in plasma with an IC_{50} of 4.67 nM. The functional TFPI capture assay used in this study could potentially be used as a pharmacodynamic marker to monitor plasma TFPI levels after the administration of anti-TFPI antibody.

REFERENCES

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Disclosures

All authors are employees of Bayer.