Selection of trigger conditions influences the effect of Factor VIII on thrombin generation in hemophilia A plasma

Poster 107-PP-W

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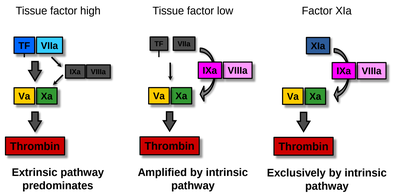
INTRODUCTION

- Measuring coagulation function is essential for monitoring hemophilia treatment. The tissue factor (TF)- or extrinsically triggered thrombin generation assay (TGA) is often utilized to monitor global hemostasis in hemophilia A.
- Sometimes, thrombin generation is initiated with factor XIa (FXIa) through the intrinsic pathway leading to higher factor VIII (FVIII) sensitivity.
- Recently, TGA has also been used to determine FVIII equivalency of an investigational bispecific antibody.

OBJECTIVE

- Studying the FVIII effect on thrombin generation using different trigger types and concentrations (Figure 1).

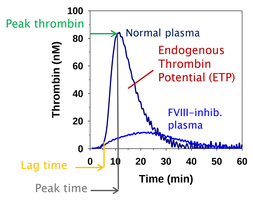
Figure 1: TGA trigger types



METHODS

- The effect of FVIII on thrombin generation was evaluated by CAT, a method described by Hemker et al. (1). For quantification of thrombin at each time point, a thrombin calibrator was included for each plasma sample.
- The CAT assay- based on the fluorogenic substrate Z-G-G-R-AMC- was performed in hemophilia A patient plasma.
- Recombinant FVIII (3-1000 mU/mL = 0.3 – 100 %) was titrated and thrombin generation triggered with TF (0.4 -20 pM) or FXIa (31 -1000 pM) and 4 µM phospholipids.
- Plasma was treated with corn trypsin inhibitor (final conc. 41.3 µg/mL; Haematologic Technologies) to inhibit undesired contact activation by factor XIIa.
- Samples were recalcified by FluCa reagent containing the substrate and CaCl2 (Thrombinoscope).
- Fluorescence was measured in a Fluoroskan Ascent® plate reader (ThermoScientific; filters 390 nm excitation and 460 nm emission) at 37°C for 90 min. All measurements were performed in duplicate.
- Thrombin levels were calculated using the calibrator, and parameters of resulting thrombin generation curves were evaluated via Thrombinoscope software (Figure 2).

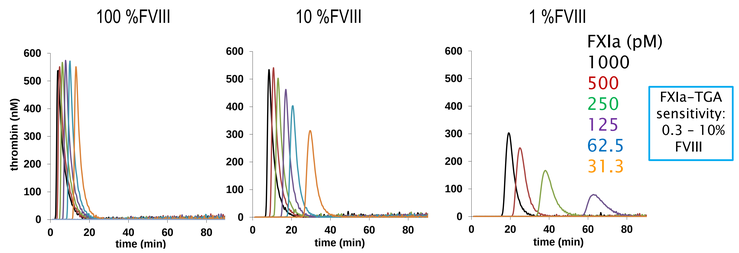
Figure 2: Calibrated automated thrombogram parameters



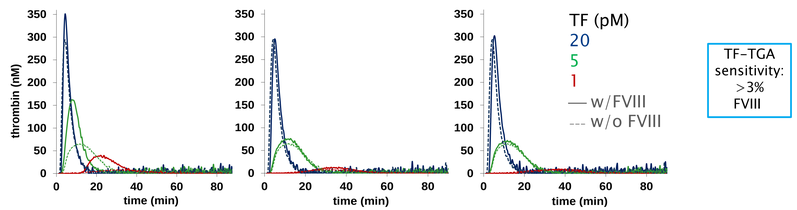
- Lag time (the initiation of thrombin generation)
- Peak time at which the maximum thrombin amount (peak thrombin) is reached
- Endogenous thrombin potential (ETP) that represents the area under the curve.

RESULTS

Figure 3: FVIII effect on thrombin generation in hemophilia A plasma



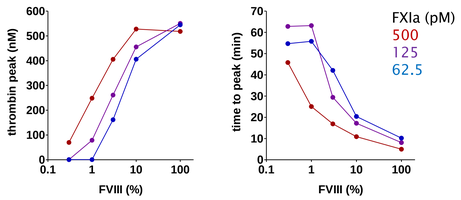
Intrinsically (FXIa) triggered TGA: Recombinant FVIII was titrated in hemophilia A patient plasma and triggered with 31.3-1000 pM FXIa . Exemplary thrombin profiles are shown for three FVIII concentrations. Without FVIII supplementation no thrombin is generated (flat line, not shown).

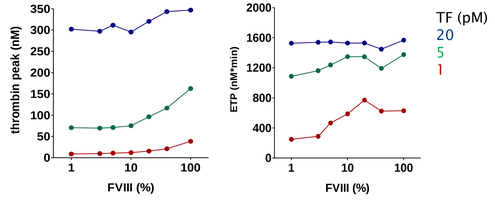


Extrinsically (TF) triggered TGA: FVIII was titrated in hemophilia A plasma and triggered with 1-20 pM TF. Exemplary thrombin profiles are shown for samples with FVIII (solid lines) and without (dashed lines).

- The type and concentration of trigger strongly influences the TGA parameters in response to FVIII. In general, TF-triggered TGA resulted in lower thrombin generation in response to FVIII than the FXIa-triggered TGA.

Figure 4: TGA parameters in response to FVIII





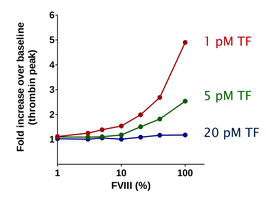
Intrinsically (FXIa) triggered TGA:

- In the FXIa-triggered TGA, the FVIII sensitivity range shifted depending on FXIa concentration. For example, the sensitive range was 1-10 % and 0.3-3 % FVIII for 125 and 500 pM FXIa, respectively.
- An increase in thrombin peak corresponded well with shortened time parameters.

Extrinsically (TF) triggered TGA:

- Thrombin peak and ETP values increased with rising TF concentrations.
- Thrombin generation at 1 pM TF was FVIII sensitive to ≥ 3% FVIII, but almost independent of FVIII at 20 pM TF.
- The time parameters changed only moderately (time to peak at 1 pM TF) or not at all (lag time; time to peak at 5 and 20 pM TF) depending on FVIII.

Figure 5: TF concentration dependency of FVIII effect



Extrinsically (TF) triggered TGA:

- Thrombin peak of sample with FVIII was expressed as fold increase over the respective sample without FVIII at the same trigger condition.
- In general, TF-triggered thrombin generation is less sensitive to FVIII with sensitivity declining at increasing TF.

CONCLUSION

- The TGA is a versatile tool to assess the hemostatic effect of FVIII in plasma from patients with hemophilia.
- Depending on trigger conditions, FVIII has a distinct effect on the TGA parameters - in different FVIII concentration ranges.
- Therefore, the kind and concentration of trigger strongly influences the assay outcome.
- This may lead to diverse results when therapeutics with distinguished mode of action are compared.

REFERENCE

1. Hemker et al., 2003 Pathophys Haemos Thromb; 33: 4-15

DISCLOSURES

All authors are full-time employees of Shire, Vienna, Austria