Evaluation of plasmin generation in severe haemophilia A patients treated with novel therapies

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Introduction

Hyperfibrinolysis has been reported to contribute to bleeding in severe haemophilia A (SHA)[1,2,3]. The impact of novel therapeutics for the treatment of HA on fibrinolysis is not known. Measuring plasmin generation (PG) may provide information on the effect of novel therapies on fibrinolysis.

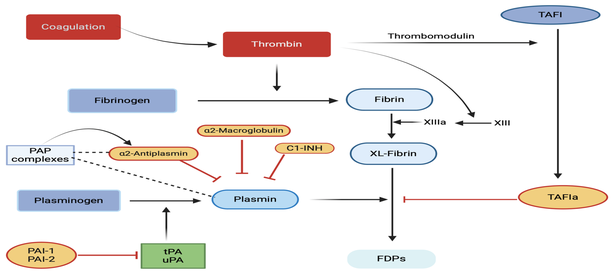

Figure 1. A schematic model of fibrinolysis (created with BioRender.com). (tPA: Tissue plasminogen activator; uPA: Urokinase-type plasminogen activator; PAI-1: Plasminogen activator inhibitor-1 (PAI-1); PAI-2: Plasminogen activator inhibitor-2; PAP complexes: Plasmin- α2- anti-plasmin; C1-INH: C-1 esterase inhibitor; TAFI: Thrombin-activated fibrinolysis inhibitor; TAFIa: Activated TAFI).

Aims

Evaluate PG kinetics pre- and post-treatment with an extended half-life recombinant factor VIII concentrate (rFVIII-EHL) and factor VIII (FVIII) bispecific antibody (FVIII-bsAb) in SHA. Correlate PG kinetics with FVIII activity and emicizumab levels.

Method

A calibrated plasmin generation assay (PGA) was performed on samples from healthy volunteers (HV; n=40), SHA patients treated with rFVIII-EHL, turoctocog alfa pegol (n=20) and SHA patients treated with FVIII-bsAb, emicizumab (n=20). PG was measured using a PG trigger reagent, plasmin calibrator and plasmin specific substrate. PG parameters: lag time (LT), endogenous plasmin potential (EPP), peak, time to peak (TtPeak) and velocity were measured. Percentage (%) normal relative to a normal pooled plasma was calculated.

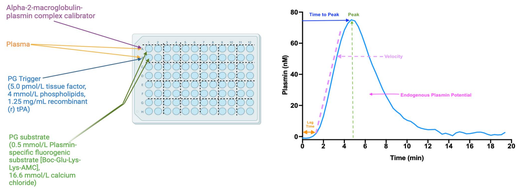

Figure 2. Calibrated plasmin generation assay (PGA) and measured PG parameters (created with BioRender.com). (PG: plasmin generation; rtPA: recombinant tissue plasminogen activator).

Results

Reference ranges (mean ± 2 SD) for PG parameters (LT, EPP, peak, TtPeak and velocity) were determined using HV samples (n= 40).

Table 1. Reference ranges for PG parameters in HV samples. Non-parametric calculation for LT (%Normal), velocity (nM/min), Velocity (%normal).

Parameters	Mean	± 2SD Range
LT (min)	2.6	1.7- 3.5
LT (%Normal)	137*	65- 208
EPP (nM•min)	241.8	88.3- 395.2
EPP (%Normal)	82	37- 127
Peak (nM)	62.5	38.2- 86.7
Peak (%Normal)	92	61- 123
TtPeak (min)	4.7	3.6- 5.8
TtPeak (%Normal)	107	82- 131
Velocity (nM/min)	30.3*	22.2- 38.4
Velocity (%Normal)	111*	78- 144

No significant differences in PG parameters was seen comparing HV and pre-treatment SHA with a baseline FVIII:C ≤5 IU/dL (n= 26). However, a significant difference (p= 0.012) in plasmin peak was observed between pre-treatment SHA samples with FVIII of <1 IU/dL (n=13) and HV samples (n=40).

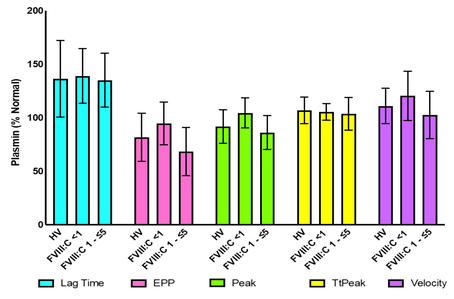

Figure 3. Figure 3. Plasmin peak parameter was significantly higher in pre-treatment SHA samples with FVIII:C Levels of <1 IU/dL than HV samples. (EPP: Endogenous plasmin potential; FVIII:C: Factor VIII activity; HV: Healthy volunteers; TtPeak: Time to peak)

A significantly reduced plasmin peak (p=0.015) and EPP (p= 0.047) were observed in steady- state FVIII-bsAb samples compared to pre-FVIII-bsAb samples. EPP was significantly higher (p=0.039) in post-rFVIII-EHL samples comparing to pre- rFVIII-EHL samples.

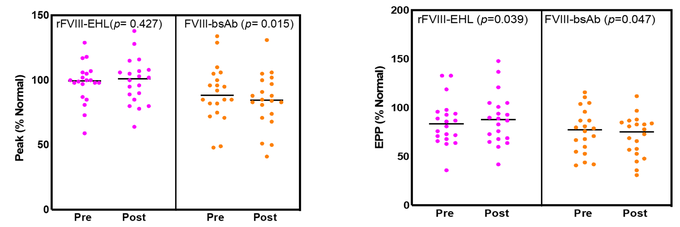

Figure 4. Plasmin peak and EPP significantly reduced in steady- state emicizumab samples. EPP significantly enhanced in post- rFVIII-EHL samples. (EPP: Endogenous plasmin potential)

A negative correlation was seen in steady- state emicizumab samples between emicizumab level and PG parameters: EPP (r= -0.515, p=0.020), peak (r= -0.469, p=0.037) and velocity (r= -0.492, p=0.027).

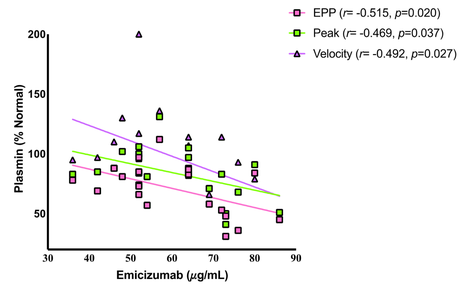

Figure 5. PG parameters (EPP, peak and velocity) showing a negative correlation with emicizumab level in steady-state emicizumab samples. (EPP: Endogenous plasmin potential

Conclusion

Pre-treatment SHA samples with FVIII of <1 IU/dL showed enhanced fibrinolysis. Novel therapies rFVIII-EHL and FVIII-bsAb impact differently on fibrinolysis. rFVIII-EHL enhanced EPP and peak whereas FVIII -bsAb suppressed PG kinetics in SHA patients.

References

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