In vitro evaluation of the effect of anticoagulation on thrombin generation in haemophilia A

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Introduction

Advancements in haemophilia treatment have extended the life expectancy of patients with haemophilia A (HA). Therefore, modern care for persons with haemophilia requires consideration of conditions associated with ageing, including thrombotic conditions that often require treatment with anticoagulation[1,2]. Currently, there is limited data from clinical trials on the effect of anticoagulant agents in HA patients treated with factor VIII concentrates or other novel therapies (e.g. FVIII bispecific antibody)[3,4].

Aim

To evaluate the in vitro effect on thrombin generation (TGT) of different anticoagulants in factor VIII deficient plasma in the presence of an extended half-life (EHL) FVIII concentrate (Elocta® / Efmoroctocog alfa - a recombinant Fc fusion FVIII) and a FVIII bispecific antibody (Hemlibra®/ Emicizumab).

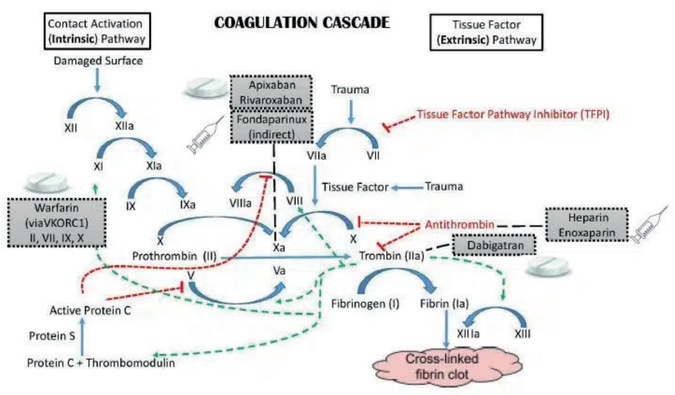


Figure 1. Coagulation cascade showing the action of DOACs (apixaban, rivaroxaban), fondaparinux and tinzaparin[5]

Methods

- 144 spiked samples were analysed in triplicate. FVIII concentrate (Elocta®) at a range of concentrations (0 to 100 IU/dL) or FVIII bispecific antibody (Hemlibra®) at 10 and 50 µg/mL were spiked into FVIII deficient plasma.
- Different anticoagulants (fondaparinux, apixaban, rivaroxaban, and tinzaparin) were then spiked into the samples above at two levels (trough and peak).
- Each sample was tested by TGT (**Figure 2**) using as the activator (trigger) recombinant human tissue factor (TF) concentrations at 5pM and 10pM (tinzaparin and fondaparinux), 5pM (apixaban and rivaroxaban) and 1pM and 5pM for the spiked FVIII bispecific antibody samples.

Results / Discussions

EHL FVIII concentrate (Elocta®) spiked apixaban and rivaroxaban

For both apixaban and rivaroxaban anticoagulants, it was noted (Figure 3):

- Using 5pM TF trigger at peak and trough levels there was TG
- Discriminatory across different Elocta concentrations
- 5pM TF trigger could be used to monitor the combined effect.

EHL FVIII concentrate (Elocta®) spiked fondaparinux, it was noted:

- Greater discrimination with 10pM TF trigger but increased variation of TGT levels using 5 and 10pM TF

EHL FVIII concentrate (Elocta®) spiked with tinzaparin, it was noted:

- For both 5 and 10pM TF triggers, at peak Tinzaparin levels, there is no TG

FVIII bispecific antibody (Hemlibra®) spiked apixaban (Figure 4) and rivaroxaban (Figure 5):

- Both 1pM and 5pM TF triggers at peak and trough levels, TG was present
- 5pM TF trigger was more discriminatory

Conclusion

TGT was discriminatory between different spiked FVIII levels and FVIII bispecific antibody samples for apixaban and rivaroxaban trough and peak levels using either TF trigger.

- Does not suggest DOAC is better.
- Spiking studies being able to test in vivo samples may confirm these project findings.

For spiked Elocta® and Hemlibra® samples containing fondaparinux and tinzaparin further optimisation of TGT conditions is required.

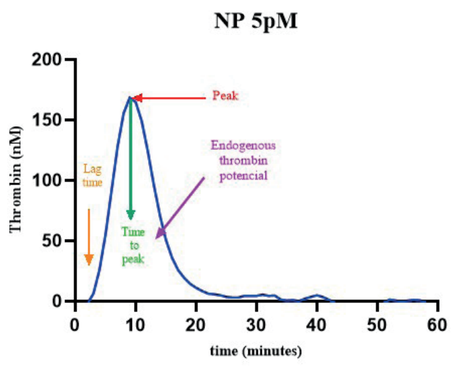
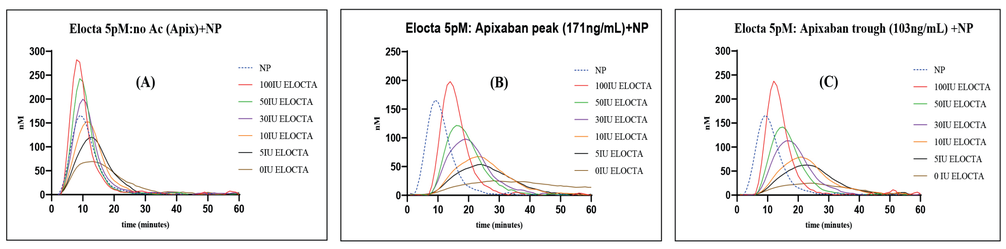


Figure 2. An example of a normal TGT curve using 5pM trigger in a lyophilised normal plasma (NP) sample.



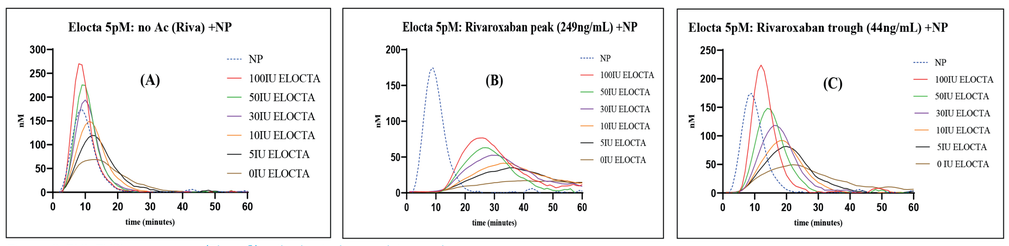


Figure 3. EHL FVIII concentrate (Elocta®) spiked apixaban and rivaroxaban

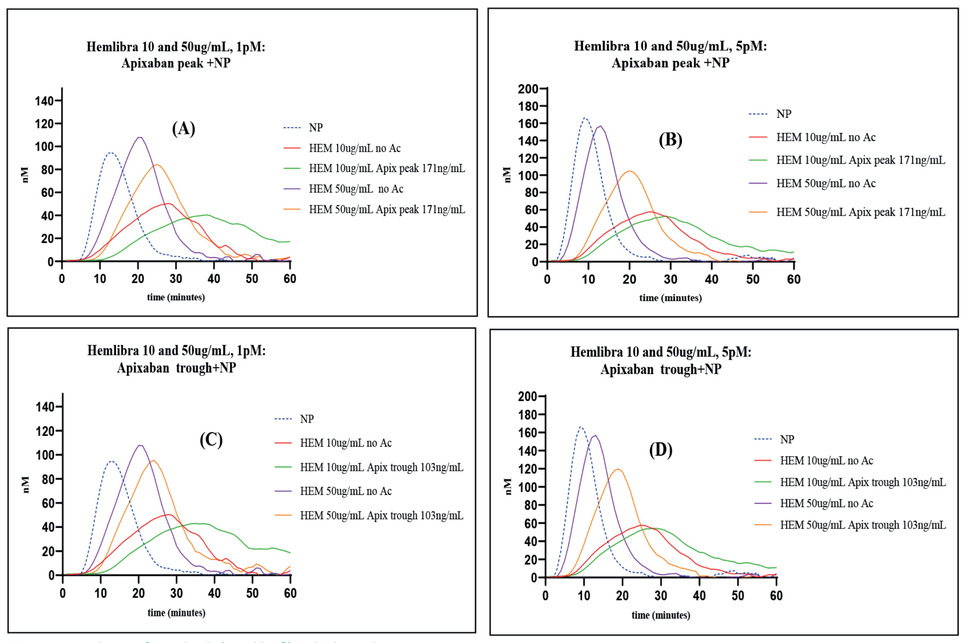


Figure 4. FVIII bispecific antibody (Hemlibra®) spiked apixaban

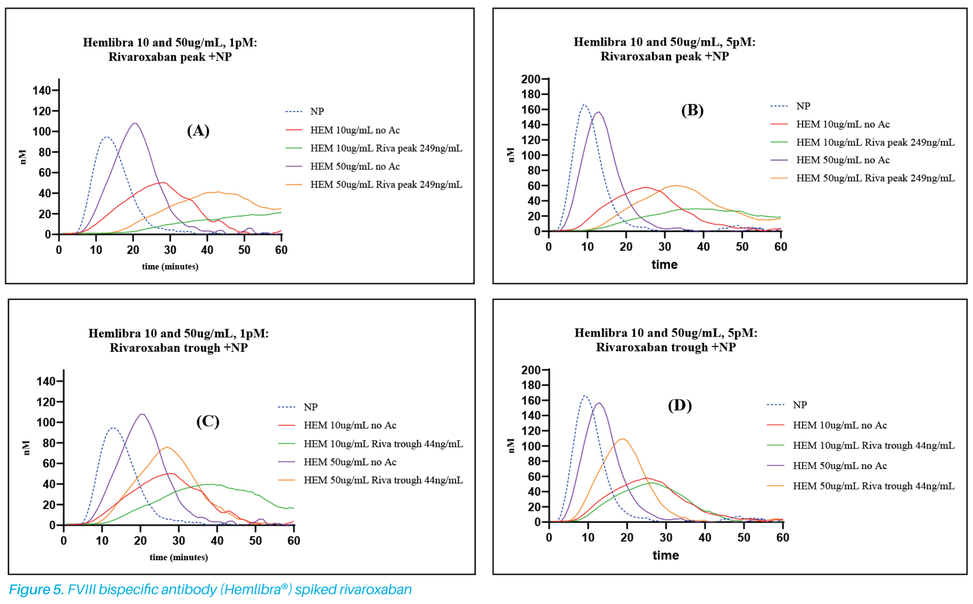


Figure 5. FVIII bispecific antibody (Hemlibra®) spiked rivaroxaban

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