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Heparin Interference of the Thrombin Generation Assay Can Be Eliminated by Heparinase Treatment

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INTRODUCTION

Reversal of anticoagulation by vitamin K antagonists (VKAs) is obtained by using four-factor prothrombin complex concentrates (PCCs). Thrombin generation assay (TGA) has been shown to be suitable for the in vivo evaluation of the hemostatic potency of PCCs. Commercially available PCCs contain heparin at different levels. Consequently, TGA results obtained for in vitro samples will be biased and may not fully reflect the clinical efficacy of PCCs observed. Removal of heparin by anion exchange adsorption has been described but this procedure could potentially alter the complex composition of PCCs. Heparin neutralization with protamine sulphate requires exact titration in order not to interfere with the determination of the hemostatic potency.

AIM

Description of a robust method to remove the inhibitory heparin effect on the TGA.

METHOD

A heparin-containing 4F-PCC sample was mixed with heparinase and incubated for 5 min at room temperature. Then, the Technothrombin TGA (Technoclone) was done using the fully automated coagulation analyzer Ceveron® (Technoclone). Normal and VKA anticoagulated plasma was used at a final heparinase concentration of 1.25 U/mL at two trigger levels. The adequate heparinase concentration was determined by investigating a concentration series of heparinase, ranging from 0.25 to 2.5 U/mL.

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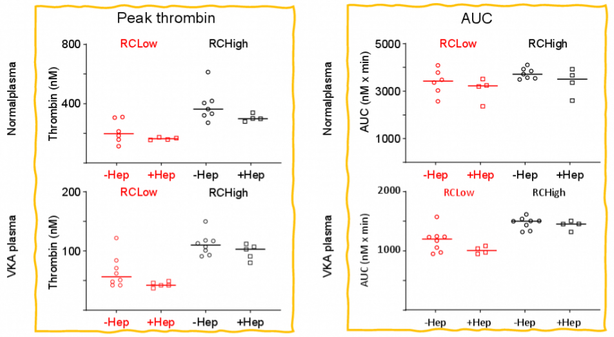
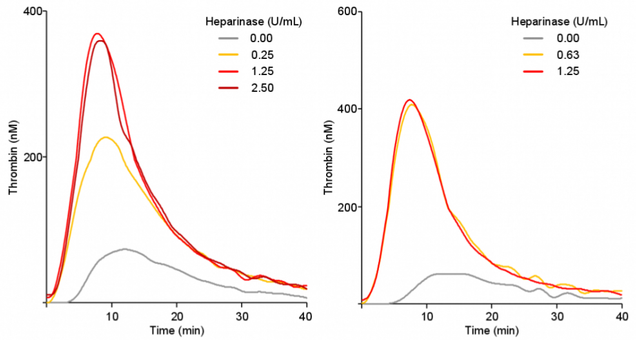
RESULTS

Influence of heparinase on TGA readouts in normal and VKA plasma

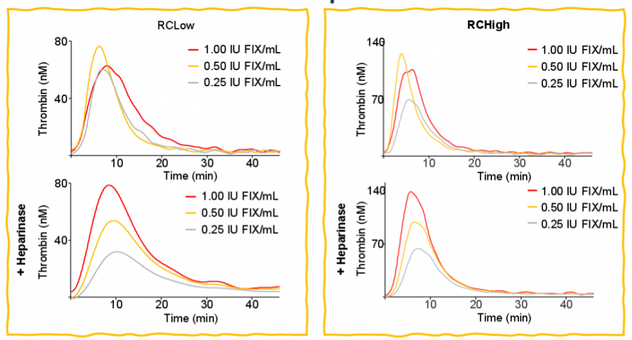


Thrombin vs time curves demonstrate the influence of heparinase

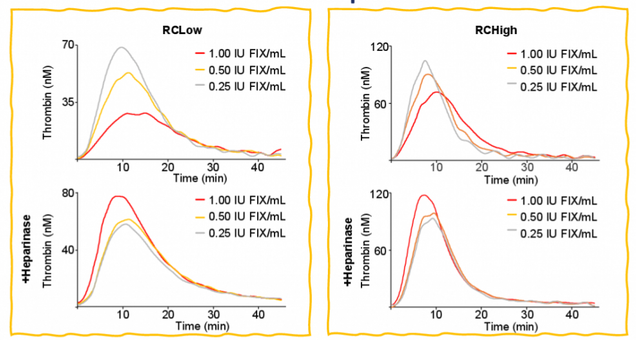


Heparinase addition at a final concentration of 1.25 IU/mL did not result in significant changes for peak thrombin and area under the curve (AUC). A heparinase level of 1.25 U/mL was shown to be as effective as 2.50 U/mL in two lots of VKA plasma, spiked with PCC, resulting in final FIX and heparin levels of 1 IU/mL and 0.38 IU/mL, respectively.

Heparinase establishes PCC dose-response in VKA plasma



Heparinase establishes PCC dose-response in normal plasma



Addition of heparinase at a final concentration of 1.25 IU/mL removes the anticoagulant activity of heparin which is present in the PCC at a concentration of 0.38 IU/IU factor IX.

CONCLUSIONS

- TGA has been shown to be a suitable method for evaluating the hemostatic potency of coagulation factor concentrates in plasma milieu.
- Commercially available PCCs contain heparin at different levels, thus TGA results will be biased by the inhibitory effect of heparin and may not fully reflect the clinical efficacy of PCCs observed.
- Removal of heparin by anion exchange adsorption has been described but could potentially alter the complex composition of PCCs, while heparin neutralization with protamine sulphate requires exact titration in order not to alter the hemostatic potency.
- Heparinase addition at a concentration of 1.25 U/mL in the final TGA reaction mix completely removed the inhibitory influence of heparin.
- The heparin-containing PCC demonstrated dose-dependent TGA response after heparinase I treatment, which was not the case when this treatment was omitted.
- Heparinase treatment can easily introduced in existing TGA test procedures and will increase the significance of data generated with TGA.