Variable Activation Kinetics of Different Recombinant Full Length and B-Domain Deleted Factor VIII Concentrates

Pahl S, Pavlova A, Driesen J, Müller J, Brondke H, Oldenburg J
Institute of Experimental Haematology and Transfusion medicine, University Clinic Bonn, Germany

Introduction

Recombinant Factor VIII (rFVIII) concentrates are used as a substitution therapy for haemophilia A patients. The concentrates differ by the length of the B domain, the cell lines used for expression (glycosylation pattern) and included polymorphisms[1,2]. This study analyses protein content, activity and thrombin activation profile of four commercially available rFVIII concentrates: Advate®, Kogenate® and Helixate® comprising a full-length FVIII protein and B domain-deleted ReFacto AF™ (Fig.1).

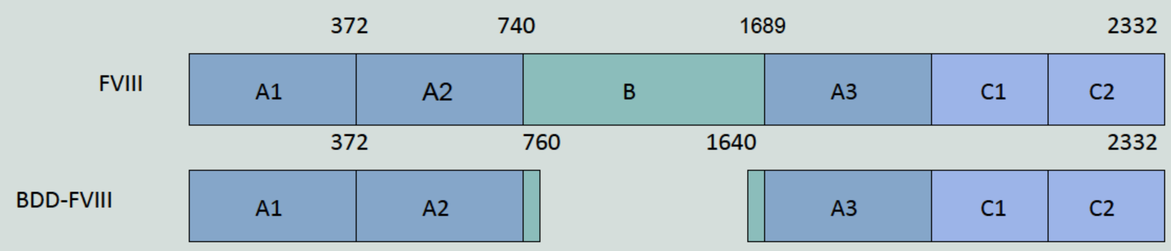

Figure 1: domain structure of the factor VIII protein with thrombin cleavage sites

Material and Methods

The recombinant full length FVIII-concentrates Advate® (Baxter), Helixate® (CSL Behring), Kogenate® (Bayer) and the B domain deleted FVIII concentrate Refacto AF® (Pfizer) were reconstituted according to the manufacturers recommendations. FVIII activity was measured by both chromogenic ($FVIII:C_{chr}$) and one-stage clotting assay ($FVIII:C_{1st}$). FVIII:Ag was quantified using both an ELISA based on polyclonal anti-FVIII capture antibodies ($FVIII:Ag_{poly}$) and another one based on monoclonal anti-FVIII light chain-IgG antibodies ($FVIII:Ag_{mono}$). The specific activity was calculated as ratio of FVIII:C and FVIII:Ag.

For analysis of the activation profile, concentrates were subjected to 1nM, 3nM or 10nM thrombin. For visualization of the A2 domain generation, western blot was performed using a primary monoclonal antibody specific for the FVIII A2 domain. Additionally, a FXa Generation Assay was performed.

Results

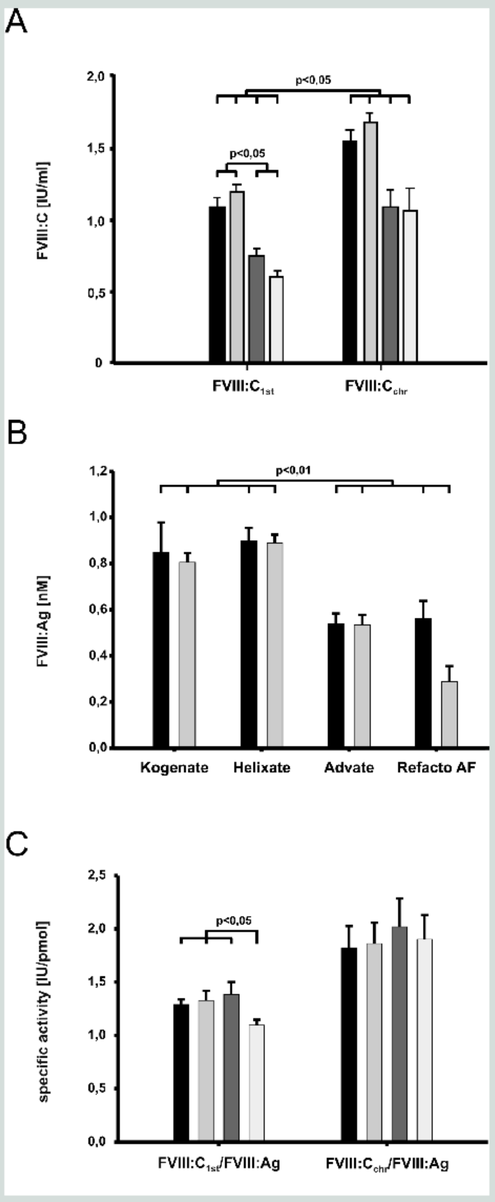

Figure 2 FVIII:C, FVIII:Ag and specific activity. A $FVIII:C_{1st}$ and $FVIII:C_{chr}$ for Kogenate® ( ), Helixate® ( ), Advate® ( ), and Refacto AF™ ( ). **B** FVIII:Ag, monoclonal ( ) or with a polyclonal ( ) capture antibody. **C** Specific activity Kogenate® ( ), Helixate® ( ), Advate® ( ), and Refacto AF™ ( ).

FVIII:C and FVIII:Ag measurements

All rFVIII concentrates show up to 40% higher $FVIII:C_{chr}$ values, in accordance with previously published data[3] (Fig.2 A).

Characteristics of Kogenate® and Helixate®, with identical formulations, were similar in all assays. However, both had significantly greater FVIII:C and FVIII:Ag relative to the measured values for Advate® and ReFacto AF™.

The calculated specific activity of all three FL-rFVIII proteins was similar regardless of the FVIII:C assay used (Fig. 2C). Interestingly, the BDDrFVIII exhibited a similar $FVIII:C_{chr}/FVIII:Ag_{mono}$ ratio, although the $FVIII:C_{1st}/FVIII:Ag_{mono}$ ratio was significantly lower compared to that for FL-rFVIII.

FVIII Activation Kinetics

The FVIII activation kinetics of the concentrates were assessed by incubation with thrombin. FVIII activation was measured by the amount of A2 generation at different time points (Fig.3).

Kogenate® and Helixate® demonstrated faster and more complete activation for all thrombin concentrations than Advate® and ReFacto AF™. With 3 nM thrombin Refacto AF™ presents the lowest rate of A2-formation and Advate® showed an activation profile in-between. The difference between Advate® and Refacto AF™ disappears with 10 nM thrombin. Changing the amount of applied FVIII protein did not influence the obtained differences (data not shown).

FXa Generation Assay

Kogenate® and Helixate® exhibited a greater *in vitro* FXa-generation than Advate® and ReFacto AF™ (Fig. 4). Importantly, we found no differences in FXa-generation among all four products when FXa-generation was normalized for FVIII:Ag.

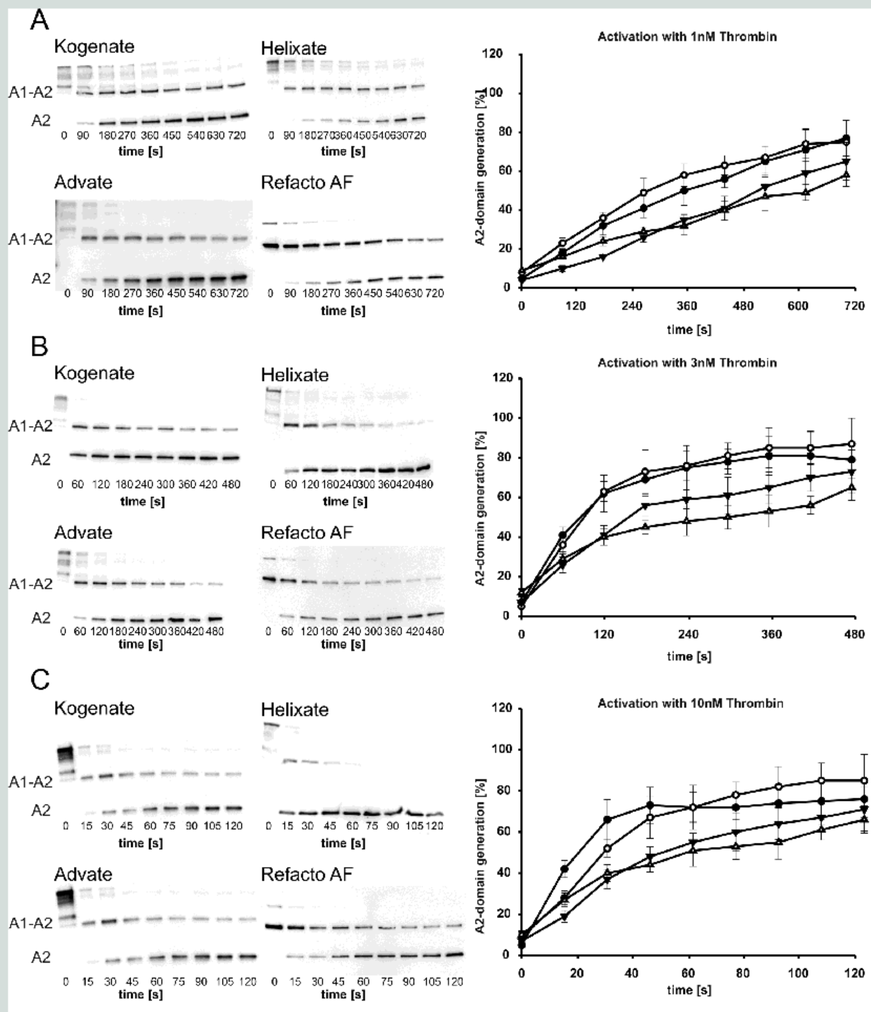

Figure 3 Time course of FVIII digestion with thrombin. Left side: Western Blot analysis of all four rFVIII concentrates at different thrombin concentrations. The first line represents the undigested FVIII at t=0 s. The A1-A2 domain fragment and the single A2 domain are indicated on the left of the Western Blots. Kogenate® ( ), Helixate® ( ), Advate® (▼), and ReFacto AF™ (▲). **A** 1 nM thrombin **B** 3 nM thrombin **C** 10 nM thrombin.

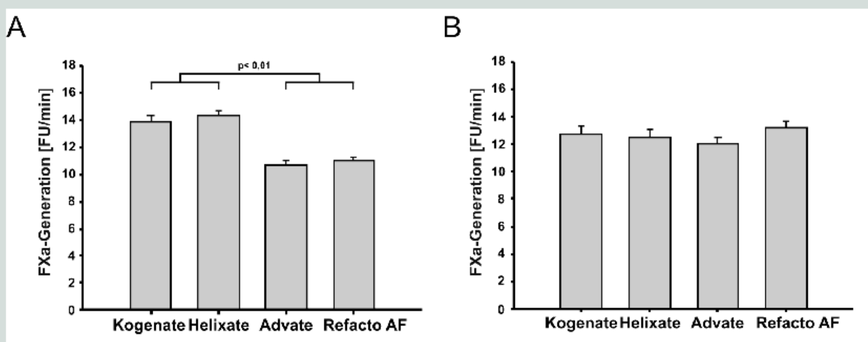

Figure 4 Results of the FXa-Generation Assay. A FVIII concentrations based on the labelled activity (1.43 IU/ml) **B** FVIII concentrations based on the antigen values (1nM).

Conclusion

The rFVIII concentrates Kogenate®, Helixate®, Advate®, and ReFacto AF™ exhibited significant differences with regard to FVIII:C, FVIII:Ag, and thrombin activation profile. The greater FVIII:C and FVIII:Ag values for Kogenate® and Helixate®, compared to Advate® and ReFacto AF™, are due to application of different standardization procedures (one-stage assay for Kogenate®/Helixate® versus chromogenic assay for Advate® and ReFacto AF™). The variation in thrombin activation profiles may arise from differences in cell line-dependent post-translational modifications of the various recombinant proteins. Furthermore, the slower activation kinetics of the BDD-rFVIII might be due in part to the absence of the B domain which may contribute to the structural integrity.

References: [1] Boedeker BG: The manufacturing of the recombinant factor VIII, Kogenate, Transfus Med Rev, 1992; **6:** 256-60 [2] Kelley et al: An improved manufacturing process for Xyntha/ReFacto AF, *Haemophilia* 2010; **16:** 717-25. [3] Mikaelsson M et al: Potency and in vivo recovery of high purity factor VIII concentrates, Thromb Haemost, 199

DOI: 10.3252/pso.eu.WFH2012.2012