The role of VWF in FVIII/VWF concentrates: biochemical characterization of three different plasma-derived factor VIII products

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BACKGROUND AND OBJECTIVES

The commercially available plasma-derived FVIII concentrates differ for purification strategies, relative content of other haemostatic proteins, stabilizers and virus inactivation strategies.This study was aimed at investigating the purity grade and biochemical properties of three commercially-available plasma-derived (pd) FVIII preparations (Emoclot®, Fanhdi® and Haemoctin®). The biochemical studies employed both chromatographic/electrophoretic characterization as well as the activation by thrombin. These studies may help to correlate the clinical efficacy with the functional and biochemical properties of the above FVIII concentrates

METHODS

Size exclusion chromatography was performed using a HiPrepTM 16/60 SephacrylTM-S500 column connected to a two-pumps FPLC apparatus. FVIII preparations before and after thrombin activation were studied by SDS-PAGE and Western blotting. FVIII activity was determined using both one-stage and chromogenic (FX activation) methods. FXa level as a function of time was calculated by a double first-order kinetic process for its generation and disappearance. Multimer pattern of VWF was accomplished by SDS-agarose gel electrophoresis with immunoblottting. VWF:Ag and VWF:act levels were measured using HemosIL AcuStar VWF:Ag and VWF:activity chemiluminescence methods (Instrumentation Laboratory, Milano, Italy). Albumin content was determined by an automated immunoassay (Hitachi 704 / Roche Albumin Reagent).

Table 1. Total protein, albumin and VWF content of PD-FVIII concentrates

Concentrate (1U/ml) [Nominal concentr.]	Total protein (mg/dL)	Albumin (mg/dL)*	VWF:Ag (%)	VWF:act (%)	Ratio (Ricof/Ag)
Emoclot	0.8	0.28	84	64	0.76
Haemoctin-pre	1.3	0.36	90	76	0.84
Haemoctin-post	1.18	0.42	88	72	0.82
Fanhdi	9	5.64	96	76	0.83

Figure 1

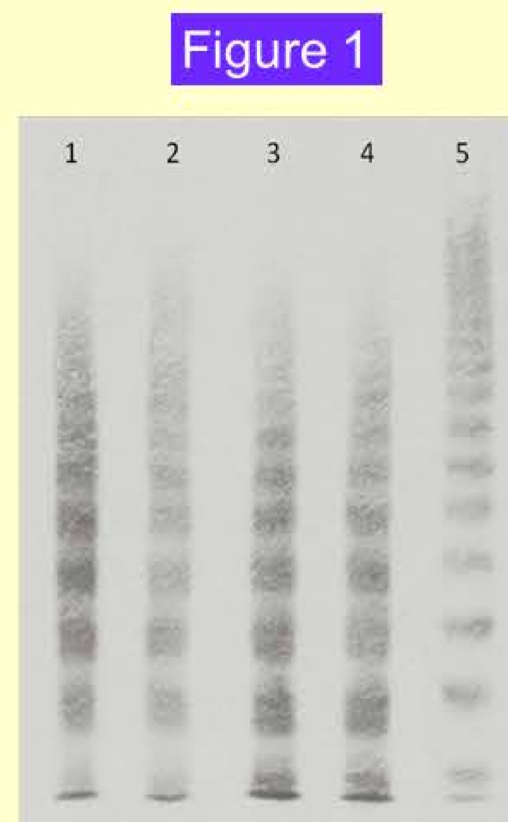

Figure 1. SDS-agarose (0.8-1.5%) electrophoresis and western blot of VWF multimers contained in different pd-derived FVIII concentrates: 1) Fahndi; 2) Hemoctin (post virucidal tretament); 3) Hemoctin (pre-virucidal treatment); 4) Emoclot; 5) for comparison, a recombinant VWF form, kindly provided by Baxter (Vienna, Austra) is also shown.

Figure 2

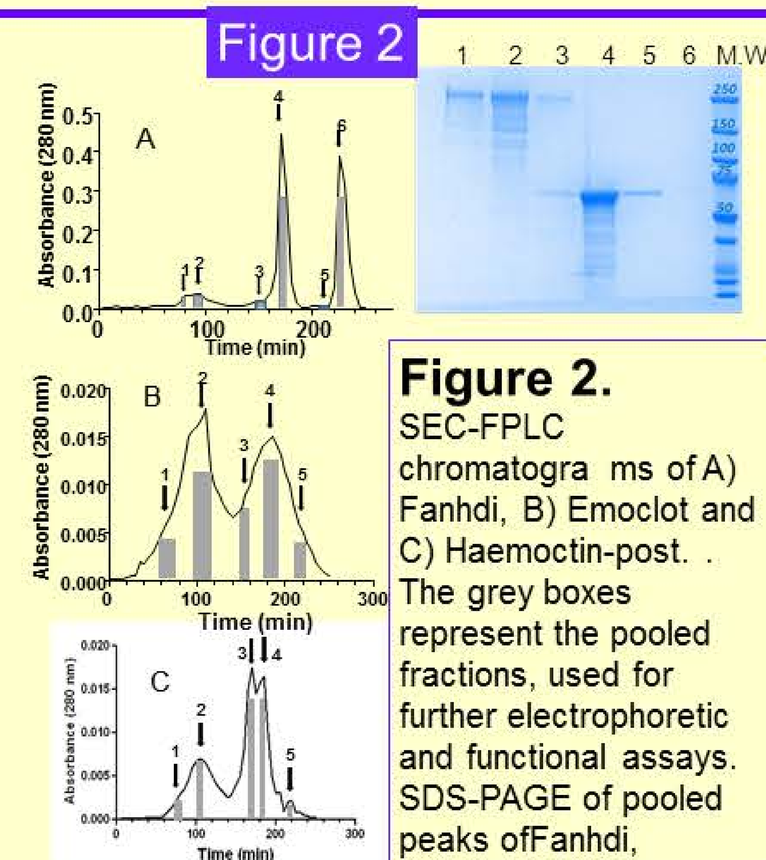

Figure 2. SEC-FPLC chromatogra ms of A) Fanhdi, B) Emoclot and C) Haemoctin-post. . The grey boxes represent the pooled fractions, used for further electrophoretic and functional assays. SDS-PAGE of pooled peaks ofFanhdi,

Figure 3

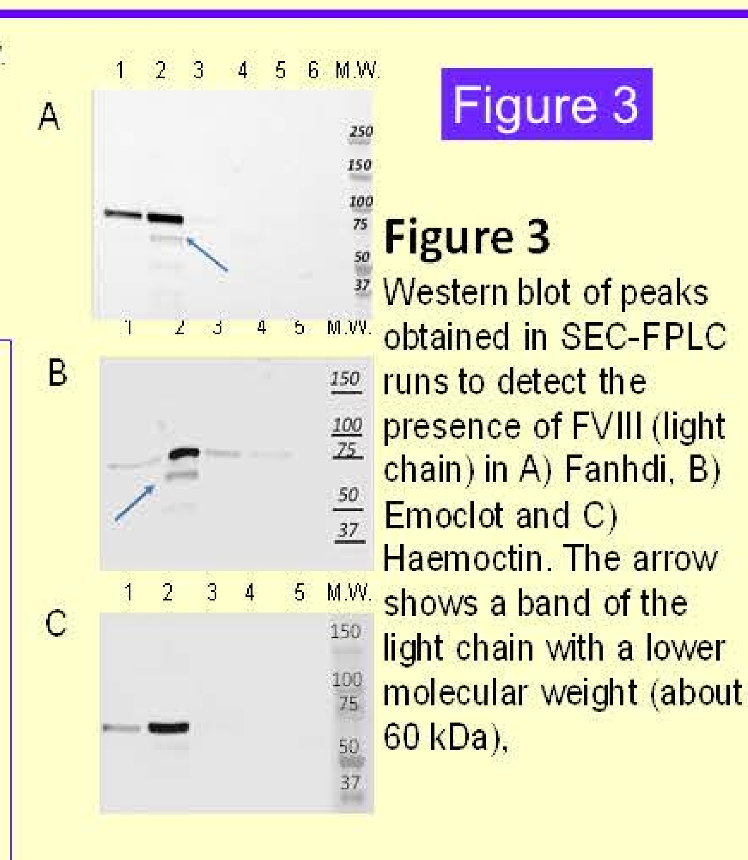

Figure 3 Western blot of peaks obtained in SEC-FPLC runs to detect the presence of FVIII (light chain) in A) Fanhdi, B) Emoclot and C) Haemoctin. The arrow shows a band of the light chain with a lower molecular weight (about 60 kDa),

RESULTS

Both total protein , albumin and VWF content of all the PD-FVIII concentrates is reported in Table 1. SDS-agarose electrophoresis and western blot of VWF multimers is shown in Figure 1. All the products presented intermediate and low molecular weight bands, whereas minor differences were observed in the high molecular weight region. SEC-FPLC runs allowed separating for all concentrates several mean peaks that were pooled and analyzed by SDS-PAGE and Western blotting. Figure 2 shows a typical chromatogram with the main peaks. The corresponding SDS-PAGE gel pertaining to Fahndi is also shown. The presence of FVIII was identified by using the MoAb sc-59512 that recognizes the light chain . As shown in the western-blot gel in Fig. 3, FVIII co-eluted with VWF multimers in fractions 1-2. Small amounts of proteolyzed light chain fragments were also identified in Fahndi and Emoclot but not in Haemoctin by bands corresponding to m.w. ranging from 37 to about 60 kDa (Fig. 3). Proteolysis and activation of FVIII by thrombin was monitored by a MoAb mapping an epitope contained in the A2 domain of FVIII. Both Fanhdi and Haemoctin produced the maximal A2 fragment of about 50 kDa approximately 15 min after thrombin addition. A complete disappearance of the A1-A2 domain (≅100 kDa band) occurred after 30 min since the reaction's start (Fig. 4A-C). Notably, after 30 min, the 50 kDa band progressively disappeared and this process was more rapid in the case of Emoclot, which already at 30 min after the reaction's start did not show this band anymore (see Fig. 4A-C). This behavior suggests that this part of the molecule was proteolytically degraded either by thrombin or other contaminating proteases associated with VWF-FVIII complex. Kinetic experiments aimed at investigating the ability of the different FVIII preparations to activate FX were analyzed by a double first-order kinetic equation. As shown in Figure 5. the best-fit parameters derived from the equation showed for all products that the value of $k_1>k_2$. Comparison the rate constants for the generation of FXa showed that Fanhdi and Haemoctin are characterized by values of k_1 higher than that of Emoclot , having, in contrast, a lower k_2 value. (see Fig. 5)

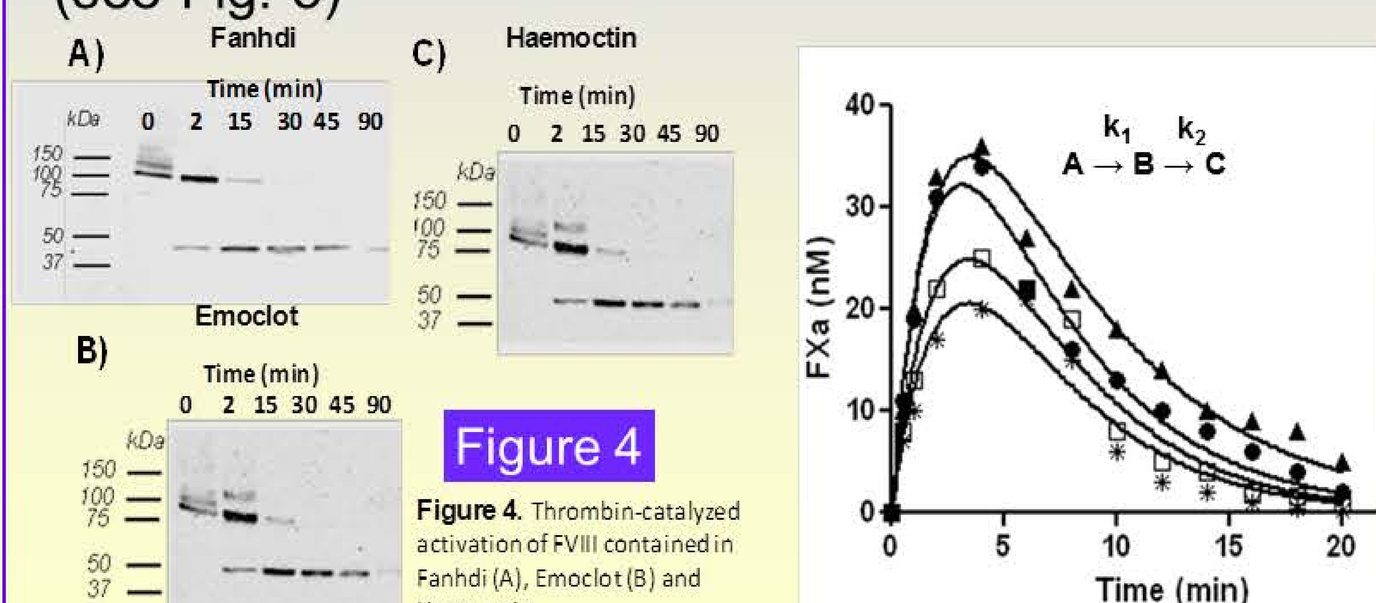

Figure 4

Figure 4. Thrombin-catalyzed activation of FVIII contained in Fanhdi (A), Emoclot (B) and Haemoctin.

Figure 5

FX activation by thrombin in the presence of FVIII of Fandhi (●), Emoclot (□), Haemoctin (▲) and Beriate P (*). The continuous lines were drawn using the best fit parameters k_1 and k_2 of eq. 2 listed in Table 3. In all cases FVIII preparations were used at 100 U/mL, whereas FX was used at 60 nM.

CONCLUSIONS

1) The three pd-FVIII concentrates contained different amounts of VWF and albumin.
2) Some concentrates contain proteolyzed light chain of FVIII
3) The global efficiency and stability of thrombin-activated FVIIIa was different and seems to positively correlate only with VWF content.

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