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ADAMTS13 content and VWF multimer and triplet structure in commercially available VWF/FVIII concentrates

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ABSTRACT

ADAMTS13 is a metalloproteinase that cleaves von Willebrand factor (VWF) into smaller multimers *in vivo*. This cleavage creates both the typical multimeric size distribution and the characteristic triplet band distribution of VWF. Here we analysed ADAMTS13 content, VWF multimeric size distribution and VWF triplet structure in five commercial VWF/factor VIII (FVIII) concentrates.

The relative distribution of ADAMTS13 activity values corresponded well to the ADAMTS13 antigen values for all examined concentrates except Haemate HS®, which had markedly higher ADAMTS13 antigen/activity ratio, with Fanhdi® and Haemate HS® displaying the most intense ADAMTS13 signal. Interestingly, ADAMTS13 levels did not correlate with the high molecular weight multimer content of the concentrates, but did correlate with VWF triplet distribution. Densitometric quantification showed that Wilate®, Immunate® and Willfact® displayed human plasma-like VWF triplet distribution, whereas Fanhdi® and Haemate HS® showed enhanced content of the faster migrating triplet band, which corresponded well to their higher ADAMTS13 content.

In summary, Immunate®, Willfact® and Wilate® had lower levels of ADAMTS13 antigen and activity and exhibited a plasma-like VWF triplet structure. Fanhdi® and Haemate HS® had higher ADAMTS13 content and an altered triplet structure. The possible impact of these observations on function and clinical efficacy of VWF/FVIII concentrates is discussed.

1. Introduction

Plasma von Willebrand factor (VWF) is a glycoprotein that mediates platelet adhesion to sites of vascular injury and is a carrier protein for blood clotting factor VIII (FVIII). VWF is synthesised in endothelial cells and megakaryocytes [1,2] and released as multimers composed of 250 kDa subunits with a molecular weight of up to >20,000 kDa. A VWF subunit carries several functional domains relevant to the role of VWF in haemostasis, including binding sites for FVIII, platelet glycoprotein Ibα (GPIbα), heparin, collagen and platelet integrin αIIbβ3 [3].

During VWF synthesis, VWF monomers are first linked together in the endoplasmic reticulum by disulphide bonds via the C-terminal cysteine knot domains to form dimers. The resulting dimers are then linked in the Golgi apparatus by disulphide bonds via their N-termini to form polymers [4]. Most of the secreted VWF is initially in the form of ultra-large VWF (ULVWF) multimers. These ULVWF multimers are converted into smaller polymers through limited proteolytic cleavage by the metalloproteinase ADAMTS13 (A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13) [5]. ADAMTS13 cleaves the Tyr1605-Met1606 bond in the VWF A2 domain [6–9]. ADAMTS13 proteolysis of multimeric VWF yields the characteristic multimer distribution found in human plasma. This cleavage also generates the slower

Abbreviations: ADAMTS13, A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13; Act, activity; Ag, antigen; BW, body weight; FRET, fluorescence resonance energy transfer; FVIII, coagulation factor VIII; GP1bα, glycoprotein Ibα; HMW, high molecular weight; Met, methionine; SHP, standard human plasma; TTP, thrombotic thrombocytopenic purpura; Tyr, tyrosine; ULVWF, ultralarge VWF (multimers); VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin cofactor activity assay.

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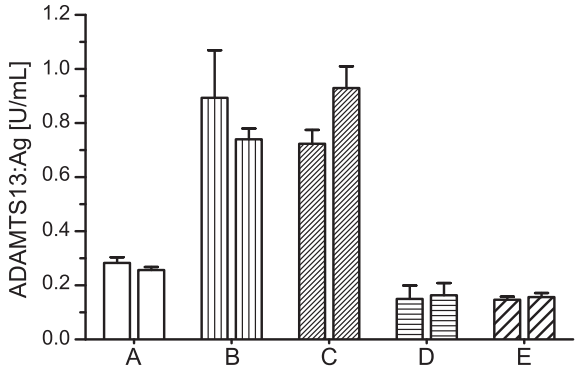

Fig. 1. ADAMTS13 antigen content of VWF/FVIII concentrates. ADAMTS13:Ag content of VWF/FVIII concentrates determined by the Technozym® ADAMTS13 antigen ELISA is shown. Mean + SD for two batches of $n = 3$ measurements each are shown. The results are given as U/mL. All values are referenced to SHP. A: Wilate®, B: Fanhdi®, C: Haemate HS®, D: Immunate®, E: Willfact®. ADAMTS13:Ag = ADAMTS13 antigen; FVIII = coagulation factor VIII; SD = standard deviation; SHP = standard human plasma; VWF = von Willebrand factor.

and faster migrating satellite bands that flank the major bands on VWF multimer-loaded gels and form the so-called VWF triplets [9].

The regulation of VWF multimer size by ADAMTS13 is critical for its physiological function [10,11]. Although it is generally accepted that VWF activity is related to its molecular weight, available data on the impact of multimeric size on VWF activity still do not allow an exact correlation and are in part conflicting [12–16]. However, it is evident that the absence of ADAMTS13 results in prothrombotic ULVWF multimers, which spontaneously bind to circulating platelets. The resulting micro-thrombi impede blood flow through smaller blood vessels, causing systemic thrombotic microangiopathies, such as thrombotic thrombocytopenic purpura (TTP) [17,18].

Although VWF cleavage by ADAMTS13 and the consequences of altered VWF multimer size distribution on VWF function have been thoroughly described, almost no attention has been paid to the ADAMTS13 content of VWF/FVIII concentrates with respect to possible VWF degradation and regarding molecular weight and triplet distribution during the manufacturing process or concentrate storage. To date, the discussion on the quality of VWF in VWF/FVIII concentrates for treatment of von Willebrand disease (VWD) has almost exclusively focused on high molecular weight (HMW) multimer content [19]. With the exception of a recent paper by Peyvandi et al. discussing the ADAMTS13 content of FVIII/VWF concentrates with respect to TTP treatment [20], almost no data are available on the residual ADAMTS13 activity and triplet band distribution in VWF/FVIII concentrates.

Triplet band distribution in VWF/FVIII concentrates might be particularly interesting as we recently demonstrated a distinct role of VWF triplet bands in glycoprotein Ib-dependent platelet adhesion and thrombus formation under flow [21], indicating that an altered VWF triplet band composition may also contribute to lower VWF-dependent platelet adhesion at high-shear flow.

Thus, we quantified ADAMTS13 activity and ADAMTS13 antigen levels in five commercial VWF/FVIII concentrates licensed for treatment of VWD. Moreover, the VWF multimer and triplet band distribution of the VWF/FVIII concentrates were evaluated by agarose gel electrophoresis and the correlation with their ADAMTS13 content was determined.

2. Materials and methods

2.1. VWF/FVIII concentrates

The following five concentrates were examined: Fanhdi® (#IBVJ201151 and GBVB1S4S81, 250 I.E.; Grifols UK, Thetford, UK); Haemate HS® (#P82666911B and P69166811B, 250 I.E.; CSL Behring, Marburg, Germany); Immunate® (#VNC3M008 and VNC3L067, 250 I.E.; Baxter, Vienna, Austria); Wilate® (#A118B181A and A213C181T1; Octapharma PPGmbH, Vienna, Austria); Willfact® (#12L08516 and 12L09581, 1000 I.E.; LFB Medicaments, Les Ulis, France). Willfact® is a pure VWF concentrate with a defined VWF ristocetin cofactor activity (VWF:RCo) of less than 10 IU FVIII/100 IU VWF:RCo. All concentrates were reconstituted according to manufacturer's instructions. Final VWF:RCo concentrations according to the manufacturers label and reconstitution volumes were 60 IU/mL for Fanhdi®, 120 IU/mL for Haemate®, 30 IU/mL for Immunate®, 100 IU/mL for Willfact® and 100 IU/mL for Wilate®. Due to the lack of an international ADAMTS13 standard, standard human plasma ([SHP]; #ORKL17, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) was used as a reference throughout all assays.

2.2. ADAMTS13 antigen assay

ADAMTS13 antigen was determined using a commercial ELISA kit (Technozym® ADAMTS-13 Antigen, Technoclone, Vienna, Austria) according to manufacturer's instructions. Measured values were calculated against the Siemens SHP calibration curve and are expressed in U/mL throughout the manuscript.

2.3. ADAMTS13 activity assay

The ADAMTS13 activity was quantified by the fluorescence resonance energy transfer (FRET) assay as described by Kokame et al. [22] using the fluorogenic substrate FRETS-VWF73 (Peptides International, Louisville, USA). Briefly, 4 μmol/L FRETS-VWF73 in buffer was added to a microtitre plate containing different VWF/FVIII concentrate samples. When this substrate is cleaved by ADAMTS13, the energy transfer that quenches the fluorescence does not occur, allowing the emission of fluorescence at 440 nm. Fluorescence was measured at +30 °C in a microplate reader (POLARstar OPTIMA, BMG LABTECH, Ortenberg, Germany) equipped with a 340 nm excitation filter and a 450 nm emission filter. Fluorescence was measured every 5 min. The reaction rate was calculated by linear regression analysis of fluorescence over time from 0 to 60 min. Increase in fluorescence is directly proportional to

Table 1
ADAMTS13 antigen and activity and respective values in relation to the VWF:RCo activity in different VWF concentrates.

Label	Concentrate	VWF:RCo[a] [IU/mL]	ADAMTS13: Ag [U/mL]	ADAMTS13: Act [U/mL]	ADAMTS13:Ag/ VWF:RCo [mU/IU]	ADAMTS13:Act/ VWF:RCo [mU/IU]	ADAMTS13:Ag/ ADAMTS13:Act [U/U]
A	Wilate®	100	0.27 ± 0.02	0.13 ± 0.03	2.70	1.29	2.10
B	Fanhdi®	60	0.82 ± 0.13	0.38 ± 0.10	13.61	6.40	2.13
C	Haemate HS®	120	0.83 ± 0.12	0.13 ± 0.07	6.89	1.12	6.14
D	Immunate®	30	0.16 ± 0.04	0.10 ± 0.04	5.22	3.21	1.63
E	Willfact®	100	0.15 ± 0.01	BDL	1.47	n.a.	n.a.

Act = activity; Ag = antigen; BDL = below detection limit (≤0.03); n.a. = not applicable; VWF:RCo = von Willebrand factor ristocetin cofactor activity.
[a] According to manufacturer's label after reconstitution.

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