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In vitro data of different factor VIII/von Willebrand factor concentrates

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A B S T R A C T

Patients with von Willebrand disease that do not respond to desmopressin should be treated with von Willebrand factor (VWF) concentrates in connection with bleedings and surgery. A recent in vitro investigation of six VWF concentrates showed large differences in composition, VWF activity and relative content of VWF and FVIII. Furthermore, different viral inactivation methods had been used. The VWF:RCo/VWF:Ag ratio ranged from 0.15-0.91, which illustrates the large differences in inactivation of VWF. This ratio correlated well with the relative amount of the high molecular weight multimers of the VWF (HMWH) in concentrates, which ranged between 15-100% of that in normal plasma. Concentrates lacking the HMWM may be less effective for mucosal bleeds. FVIII is more important for surgical hemostasis. In this study the FVIII/VWF:RCo ratios varied considerably between 0.02-6. Concentrates with a high VWF/FVIII ratio may induce very high levels of FVIII in patients, as endogenously released FVIII adds to the infused FVIII. The concentrate that was almost devoid of FVIII should be given 12-24 hours before surgery in order to allow the endogenously released FVIII to increase sufficiently, or be combined with a FVIII concentrate. It is important to be aware of the differences between the concentrates as it may have significant clinical implications.

Key words: Willebrand, FVIII, factor concentrates, *in vitro.*

Treatment of von Willebrand disease (VWD) aims at normalizing the activity of von Willebrand factor (VWF) and factor VIII (FVIII) in plasma. In patients that are non-responders to desmopressin, this can be achieved by intravenous infusion of a concentrate containing VWF. Several concentrates are available worldwide that may be used for treatment of VWD because they contain VWF. Some of them were primarily manufactured for treatment of hemophilia A and therefore also contain FVIII. In some, the VWF may have been denatured due to the manufacturing process. This may result in a lack of the largest multimers of the VWF, probably due to proteolytic breakdown. Concentrates lacking the high molecular weight multimers (HMWM) of the VWF are probably insufficiently effective for treatment of mucosal bleedings, whereas FVIII is most important for treatment of surgical bleedings.[1] It is, however, not sufficient to treat VWD patients with a pure FVIII concentrate, as FVIII has a very short half-life when it is not protected by VWF in plasma.[2] The ratio between VWF and FVIII in the concentrates is important to know, as infusion of large amounts of FVIII to VWD patients may induce very high plasma levels,[3] since VWD patients release endogenous FVIII when VWF levels in plasma are normalized. A pure VWF concentrate may be used, but in acute situations it has to be combined with a FVIII concentrate as the endogenous FVIII release is slow and reaches maximum levels after about 24 hours.[2]

In vitro evaluation of VWF concentrates

The in vitro characteristics of six VWF containing concentrates were recently published (Table 1).[4] All six concentrates had undergone viral inactivation. The specific activity (VWF:RCo/total amount of protein) varied considerable between the concentrates (5-130 IU mg-1) (Figure 1). The specific activities were comparable to those found by others.[5] A low specific activity reflects a large content of contaminating proteins. Efforts should made to improve the purification of VWF concentrates, as such impurities may cause side-effects, e.g. haemolytic transfusion reactions[6] or allergic reactions. The ratio between VWF activity and VWF antigen can be used as a meas-

Table 1. List of VWF concentrates included in the *in vitro* investigation.[4]

Concentrate	*Company*	*Virus inactivation*	*Label on vials and content*
Immunate	Baxter Bioscience, Germany	Tween 80 Vapor heating 60°C, 10h	FVIII:C 1000 IU/10mL
Factor Willebrand	LFB, France	Solvent/ Detergent (TNBP/polysorbate 80)	VWF:RCo 1000 IU/20 mL
Innobrand	LFB, France	Solvent/ Detergent (TNBP/tween)	VWF:RCo 1100 IU/20mL
Haemate P	Aventis Behring, Germany	Pasteurization 60°C, 10h	FVIII:C 250 IU/10mL
8Y	BPL, UK	Dry heat 80°C, 72h	FVIII:C 315 IU/10mL
Koate DVI	Bayer Corp, USA	Solvent/ Detergent + Dry heat 80°C, 72h	FVIII:C 1050 IU/10 mL

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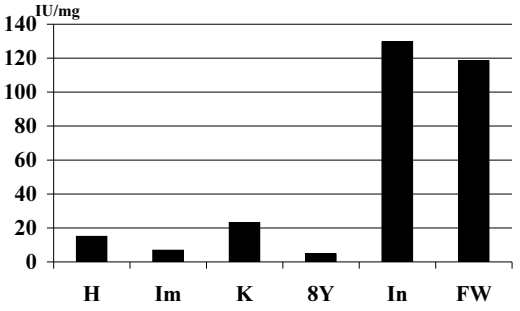

Figure 1. The specific activity (IU/mg) in six VWF concentrates measured as the ratio between the VWF activity (VWF:RCo) and total protein content. The concentrates are: H=Haemate, Im=Immunate, K=Koate, 8Y, In=Innobrand and FW=Facteur Willebrand.[4] *This research was originally published in the journal Haemophilia. ©2004 Blackwell Publishing Ltd.*

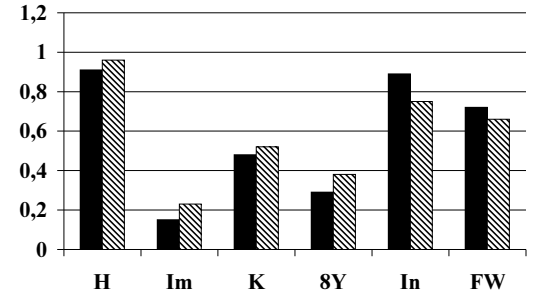

Figure 2. The VWF:RCo/VWF:Ag (black bars) and VWF:CB/VWF:Ag (striped bars) ratios in six VWF concentrates: H=Haemate, Im=Immunate, K=Koate, 8Y, In=Innobrand and FW=Facteur Willebrand.[4] *This research was originally published in the journal Haemophilia. ©2004 Blackwell Publishing Ltd.*

ure of the degree of inactivation of VWF. By definition, the ratio should be about 1.0 if VWF function is normal. A low ratio indicates loss of function. This is in parallel with patients in whom a ratio of ≥0.7 is seen in patients with type 1, whereas a ratio of < 0.7 indicates that the patients have a functionally defective VWF and thus a type 2 variant. VWF activity can be measured with a ristocetin cofactor activity method (VWF:RCo) or a collagen binding assay (VWF:CB). There was a good agreement between the two activity methods in this study. In contrast, there was a poor correlation between the activity methods and the VWF:Ag which reflects a varying degree of inactivation of VWF in the different concentrates. The mean VWF:RCo ratio was 0.55 (SD 0.30) and the mean VWF:CB ratio was 0.57 (SD 0.24) (Figure 2). Only three concentrates (Haemate-P, Innobrand and Facteur Willebrand) had ratios >0.7.

We also tested the multimeric composition with SDS-agaros gel electrophoresis followed by immunoblotting and densitometry. None of the concentrates had a completely normal multimeric composition, but there were large differences between concentrates. The densitometric evaluation gave an objective measure of the amount of large multimers that were present in the concentrates as compared to normal plasma. Three concentrates (Haemate-P, Innobrand and Facteur Willebrand) had a relative content of large multimers (HMWM) close to that in plasma (Figure 3).

There was a good correlation between the two methods of comparing loss of VWF function, i.e. the VWF:RCo/VWF:Ag ratio and the amount of HMWM as measured by densitometry (R=0.96, p=0.0006), which compares well with earlier findings.[7]

The ratio between FVIII and VWF in the concentrates is important to know, as infusion of both FVIII and

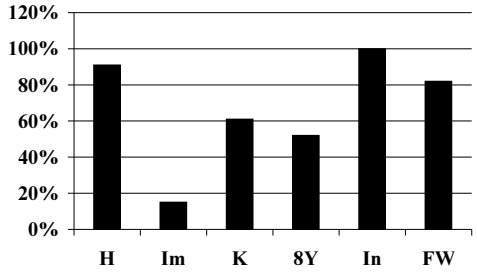

Figure 3. Densitometric analysis of the multimeric composition of the VWF in six concentrates tested with SDS-agaros gel electrophoresis. The concentrates are: H: haemate; Im: immunate; K: Koate; 8Y, In: Innobrand and FW;: facteur Willebrand (4). Values on Y-axis refer to relative content of HMWM above the 7th multimer in percent of that in normal plasma. ***This research was originally published in the journal Haemophilia. © [2004] Blackwell Publishing Ltd.***

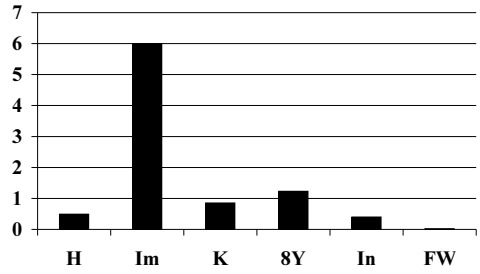

Figure 4. The FVIII:C/VWF:RCo ratios in six VWF concentrates: H: Haemate; Im: Immunate; K: Koate; 8Y, In: Innobrand and FW: facteur Willebrand (4). ***This research was originally published in the journal Haemophilia. © [2004] Blackwell Publishing Ltd.***

VWF may lead to very high plasma levels of FVIII, which may induce a risk of thrombotic complications. There is no certain evidence of a relation between thrombosis and factor infusion in VWD, but there is a relation between high FVIII levels and thrombosis in population studies. It is therefore advisable to avoid very high plasma levels of FVIII. The relative content of FVIII and VWF varied considerably between the concentrates with a FVIII:C/VWF:RCo ratio between 0.02 and 6 (Figure 4). The concentrate with the lowest ratio (Facteur Willebrand) is almost free of FVIII. This can be advantageous when repeated doses are given over longer periods. The infused VWF induces an endogenous release of FVIII, with maximum FVIII levels in plasma after about 12-24 hours. In an acute situation, therefore, a FVIII-free VWF-concentrate must be combined with a FVIII concentrate.

Summary

There are considerable differences in the in vitro content of different VWF concentrates. The composition of a concentrate is important both for the efficacy in different kinds of bleeding and for the risk of side effects. Concentrates lacking the largest multimers and having a low activity of the VWF as compared to VWF antigen content are probably less effective for treatment of mucosal bleedings. Concentrates with a low specific activity (VWF:RCo/total protein) may theoretically cause an increased risk of hemolytic or allergic reactions. Concentrates with a high FVIII:C/VWF:RCo ratio may induce very high FVIII levels, whereas those lacking FVIII should be given 12-24 hours before a hemostatic challenge or be combined with a FVIII concentrate. It is important that the treating physicians are aware of the differences between concentrates in order to avoid side effects or insufficient effect. Therefore both VWF and FVIII activities should be labeled on the concentrates that are intended for use in VWD.

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