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

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.
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 $\geq 0.7$ 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.

Table 1. List of VWF concentrates included in the in vitro investigation.†

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

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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.† This research was originally published in the journal Haemophilia. ©2004 Blackwell Publishing Ltd.

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.†

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

**References**


