ADAMTS13 in von Willebrand factor (VWF) concentrations:
Is an intact VWF triplex structure important for VWF function?

**Introduction**

Plasma von Willebrand Factor (VWF) mediates platelet adhesion at sites of vascular injury and functions as carrier protein for blood clotting factor VIII (FVIII). The glycoprotein is synthesized in endothelial cells and megacaryocytes and released into plasma as multimers composed of 250 to 12,000 subunits with molecular weights ranging up to 20 x 10^6 kDa. The characteristic multimer pattern of plasmatic VWF results from asymmetric cleavage by the processing proteases ADAMTS13 between N1605/M1606 within the VWF A2 domain (see Fig. 1). In normal plasma, characteristic species of various multimeric sizes with spanning satellite bands (triplets) encompassing the major band on VWF multimer gels are present. The faster and slower migrating band groups accompanying a VWF multimer lack one Nterminal fragment or an additional N-terminal fragment, respectively. Defects in the secretion of VWF impaired assembly of VWF multimers, or increased proteolytic VWF degradation causes von Willebrand Disease (VWD). Distribution of VWF satellite bands is significantly altered in some types of VWD and in different plasma-derived (pd) VWF / FVIII concentrates. Depletion of ADAMTS13 during plasma fractionation is not yet fully explored, but at least part of the plasmatic protease may be present in the cryoprecipitate. Depending on the efficacy of the individual production processes to remove ADAMTS13, it is assumed to be found in different amounts in VWF / FVIII products. However, the impact of ADAMTS13 content on triplex structure and its effect on VWF function has not been investigated so far.

We quantified ADAMTS13 activity and antigen levels in the VWF / FVIII concentrates. ADAMTS13 activity and antigen analysis: Alterations of VWF triplet distribution were determined using agarose gel electrophoresis. VWF multimeric plasma like VWF multimer pattern and triplet structure using HAC with salt step gradient elution, followed by size exclusion chromatography (HAC). VWF-mediated platelet adhesion after flow was determined using a flow chamber model.

**Materials and Methods**

VWF / FVIII concentrates (wilate®, Octapharma PPGmbH, Austria; concentrate A: plasmatic VWF results from asymmetric cleavage by the processing proteases ADAMTS13 and released into plasma as blood clotting factor VIII (FVIII). The glycoprotein is synthesized in Fuchs B, Solecka B, Kröning M, Kannicht C Octapharma R&D, Molecular Biochemistry Berlin, D-14195 Berlin, Germany

**Results**

The values of the analyzed concentrate samples varied between 2 mU and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentrate A and 20 mU ADAMTS13/IU FVIII:C. Analysis of several lots of the high-purity concentran...