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Full Length Article

Acquired von Willebrand factor deficiency caused by LVAD is ADAMTS-13 and platelet dependent☆

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ABSTRACT

Introduction: The high shear rates induced by left ventricular assist devices cause acquired von Willebrand disease (aVWD). We hypothesised that an ex vivo model could be established to study whether mechanical shear stress alone causes aVWD or whether this process depends also on the VWF cleavage protein ADAMTS-13 and on platelets.
Materials and methods: Healthy volunteers and two patients with congenital ADAMTS-13 deficiency donated blood. In vitro closed extracorporeal circuits were established using medically approved left ventricular assist devices (LVAD). VWF multimers were quantified by gel electrophoresis; VWF antigen, ristocetin cofactor activity (VWF:RCo), ADAMTS-13 levels and platelet function were assessed.
Results: The high shear stress in the extracorporeal circulation rapidly decreased VWF:RCo and thereby the VWF:RCo/VWF:Ag ratio by 47% ($p < 0.01$) to pathologically low values. Concomitantly, high molecular weight multimers (HMWM) decreased: up to 14–15 mers were visible on the gels at baseline, which were reduced by a maximum of 6–7 mers, corresponding to an average 68% lower densitometry signal of HMWM ($p < 0.001$). This was accompanied by marked reduction of aggregation by various agonists ($p < 0.005$). In contrast, the two patients with congenital thrombocytopenic purpura with virtually complete deficiency of ADAMTS-13 activity had only a minimal or no decrease in multimers ($p < 0.005$ vs. healthy controls). Similarly, no or minimal depletion of large multimers occurred, when normal plasma circulated without platelets.
Conclusion: An in vitro model for LVAD associated aVWD demonstrated that ADAMTS-13 and platelets contribute to the depletion of HMWM of VWF.

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1. Introduction

von Willebrand factor (VWF) is a high molecular weight glycoprotein that mediates platelet adhesion at the site of vascular injury, especially under high fluid shear conditions [1]. VWF is secreted from endothelial cells as ultra-large multimers which are processed into smaller regular sized multimers through enzymatic cleavage by the metalloprotease ADAMTS 13 thereafter [2]. The larger VWF multimers are the most haemostatically competent [3], and the loss of these is associated with the severe bleeding complications as seen in patients with type 2A von Willebrand disease (VWD). Apart from congenital VWD, acquired type 2A VWD may arise in patients with aortic stenosis [4–7] or patients with left ventricular assist devices [8]. On the other hand, loss of VWF multimer size regulation caused by severe ADAMTS-13 deficiency (as observed in patients with thrombotic thrombocytopenic purpura) leads to enhanced aggregation of platelets on ultralarge-VWF multimers and consequently to microangiopathic haemolytic anaemia and thrombocytopenia.

Use of left ventricular assist devices (LVADs) for treating end-stage heart failure is becoming more and more important for patients awaiting heart transplantation [9]. Furthermore, LVADs are also increasingly implanted in patients as destination therapy, when heart transplantation is not considered as an option [10]. Artificial surfaces and the high blood shear rates of LVADs induce activation of coagulation and of the fibrinolytic system [11]. As thromboembolic events are

Abbreviations: aVWD, acquired von Willebrand disease; VWF, von Willebrand factor; LVAD, left ventricular assist device; VWF:Ag, von Willebrand factor antigen; VWF:RCo, ristocetin cofactor activity; HMWM, high molecular weight multimers; LMWM, low molecular weight multimers; PRP, platelet rich plasma.

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frequent with these devices, the patients require adequate anticoagulation [12,13]. A combination of platelet inhibitors and a vitamin K antagonist is often used in these patients [14]. Also, bleeding complications, mainly gastrointestinal bleeding, have been reported in left ventricular assist device recipients [15], which cannot be solely explained by the anticoagulation therapy. Furthermore, markedly impaired platelet function was observed in outpatients with left ventricular assist devices [8]: platelet function under high shear rates was severely compromised, ristocetin-induced platelet aggregation was also reduced. Although von Willebrand factor antigen levels (VWF:Ag) were 80% higher than in healthy individuals, subnormal VWF activity, measured as ristocetin-cofactor activity (VWF:RCO) levels were found in some patients. Western blot analysis of VWF multimers revealed an abnormal VWF multimeric pattern: degradation of high molecular weight multimers (HMWM) was accompanied by enhanced triplet structure and increased low molecular weight multimers (LMWM). This picture resembles type 2A von Willebrand disease. Acquired von Willebrand disease was also confirmed in patients with two different types of LVAD [16], and a lower VWF:RCO/VWF:Ag ratio was associated with larger transfusion requirements.

We hypothesised that an ex vivo model could be established to study whether mechanical shear alone causes an acquired VWD 2A or whether this process also depends on the VWF cleavage protein ADAMTS-13. Therefore we used blood from healthy volunteers and two stable patients with congenital thrombotic thrombocytopenic purpura (TTP). Further, we wanted to know whether platelets are essential for the cleavage of the large multimers during LVAD circulation. Thus, we compared the effect of circulating platelet rich plasma (PRP) to circulating platelet poor plasma on the multimeric pattern of VWF.

2. Material and methods

2.1. Study design and subject population

The Ethics Committee of the Medical University of Vienna approved the study protocol, and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all healthy volunteers and patients before the study entry.

Twelve healthy male and female volunteers (m/f ratio 6/6; age 23 years, range: 18–59, body mass index 24 ± 34.2 kg/m^2; mean ± standard deviations) and two patients with congenital ADAMTS-13 deficiency were included in this study, one of whom is on biweekly treatment with plasma infusion. Healthy subjects were excluded if they had abnormal findings in medical history and physical examination unless the investigator considered this abnormality clinically irrelevant. Seropositivity for hepatitis B virus surface antigen, hepatitis C virus or human immunodeficiency virus-1/2 antibodies, blood donation during one month prior to the study, pregnancy and use of medication during two weeks before the start of the study (which the investigator considers may affect the validity of the study) precluded a subject from being enrolled.

Healthy volunteers underwent a single whole blood donation of approximately 240 mL. Vital parameters were measured before and after blood withdrawal. The two female congenital TTP patients, who were described in more detail previously in another trial [17], also underwent blood donation (240 mL). Blood was sampled in citrate dextrose (1:9) containing bags.

2.2. Ex vivo experiments

A hydraulic circuit was established using a medically approved left ventricular assist device (Heartware HVAD®, Heartware Inc., Miami Lakes, FL) connected to a fluid reservoir by medical grade silicone tubings (Degania Silicone Ltd., Israel) and a medical grade polyvinylchloride (PVC) tubing (Tygon® S50 HL, Saint-Gobain Performance Plastics, Akron, OH) (see Supplementary Fig. 2 for setup of circuit). The reservoir was a custom made thin-walled silicone tubing (wall thickness 0.6 mm) out of casting silicone (Köraform A42, Kömmerling Chemische Fabrik GmbH, Germany). This allowed oxygenation of the blood by ambient atmosphere during the test. Paired tests with two circuits were performed at 37 °C in an incubator (Duomax 1030T/Inkubator 1000, Heidolph Instruments GmbH & Co.KG, Germany). The priming volume of the setup with 90 mL allowed the use the blood of one donation in both circuits. Apart from the pumps, all blood contacting parts were exchanged for each experiment. The pumps were cleaned immediately after the tests by running for 1 h in alkaline cleaning fluid (S + M® Labor, Schülke & Mayr GmbH, Vienna, Austria), for 24 h in instrument disinfectant (Sekusept® plus, Ecolab Deutschland GmbH, Düsseldorf, Germany) and for 24 h in deionized water. Pump speed was set to 1800 rpm and circulating blood volume to 5 L/min by adjusting the flow resistance, to correspond to approximately the in vivo use in humans. We had also applied higher pump speeds (up to 3000 rpm) in first experiments, but the circulating blood was too haemolytic for further analysis. Pressure was measured at the pump inlet and the pump outlet by a disposable pressure transducer (TruWave Pressure Transducer, Edwards Lifesciences Corp., Irvine, CA). Citrate phosphate dextrose solution (1:7) or heparin (5 U/mL) anticoagulated blood circulated for 2 h in the extracorporeal circuit. As shown in a recent study [18], we could not detect any difference between the two anticoagulants in terms of any tested parameter (VWF multimers and assays). Two circuits were run in parallel for whole blood experiments and the blood from patients with congenital TTP; the reduction in multimer bands was comparable between both circuits and the difference in the number of multimer bands was 0–1 bands in all cases. A mean was taken for statistical comparisons.

2.3. Laboratory analysis

VWF:Ag was measured with a fully automated simultaneous thermal analyser using the STA Liatest VWF (Diagnostica Stago, Paris, France). VWF:RCo was assayed by turbidometry using a commercial kit (BC von Willebrand reagent; DadeBehring, Marburg, Germany) [19].

Determination of VWF multimers was performed on sodium dodecyl sulphate-agarose discontinuous electrophoresis (1.2% agarose gel) using LGT agarose type VII (Sigma, Munich, Germany). The VWF multimers were transferred to nitrocellulose filters by electroblotting. Filters were incubated at room temperature in a 1:3000 dilution of polyclonal rabbit anti-human VWF–horseradish peroxidase antibody (Dako, Glostrup, Denmark). After several washing steps, SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL) was added to the filters. Detection of multimers was performed by using the luminescent image analyser LAS 3000 (Fujifilm Life Science, Duesseldorf, Germany).

The ADAMTS-13 activity was determined by a commercially available assay (Technozym ADAMTS-13 activity ELISA; lower limit of quantification: 0.35%). Plasma samples were diluted 1:1 and 1:2 prior to analysis as duplicates. ADAMTS-13 antigen levels were determined by a commercially available assay (Technozym ADAMTS-13 Antigen ELISA); plasma samples of healthy volunteers were diluted 1:5 prior to analysis.

Platelet function was measured in healthy volunteers by the Multiplate analyser (MEA; Multiplate; Dynabyte Medical, Munich, Germany), as previously described [20]. Blood was drawn into tubes containing hirudin as an anticoagulant and diluted with saline solution (0.9%) at a 1:1 ratio and incubated for three minutes. After stirring at 37 °C, platelet agonists, ADP (6.5 μM), arachidonic acid (0.5 mM), ristocetin (0.77 mg/mL) and TRAP-6 (32 μM) were added and aggregation was continuously recorded for 5 min. Aggregation was quantified as area under the curve (AUC), an integrated measure of velocity and maximal aggregation, and expressed as units (U).

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