Pathophysiology and management of hemostatic alterations in cirrhosis and liver transplantation
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CHAPTER 2

Infusion of DDAVP does not improve primary hemostasis in patients with cirrhosis


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ABSTRACT

Background: Cirrhosis frequently affects multiple components of hemostasis. Reversal of the coagulopathy of these patients is frequently required in case of bleeding episodes, or as prophylaxis before invasive procedures. Although 1-deamino-8-D-arginine vasopressin (DDAVP) is widely used as a pro-hemostatic agent in patients with cirrhosis, it is unclear whether DDAVP truly enhances hemostasis in these patients. Here we investigated the hemostatic effects of a single bolus of DDAVP in patients with cirrhosis.

Methods: Ten patients with cirrhosis (child B or C) and ten patients with mild hemophilia A received an intravenous single bolus of 0.3 microgram/kg DDAVP. Plasma was collected prior to and at 1, 3, 6, and 24 hours after DDAVP administration. Levels of Von Willebrand factor (VWF), VWF propeptide, factor VIII (FVIII), and ADAMTS13 were measured in all plasma samples, whereas VWF multimers and functional VWF-dependent platelet adhesion were determined in the samples pre- and 1 hour after DDAVP administration.

Results: Following DDAVP administration, VWF, FVIII, and VWF propeptide levels increased in patients with hemophilia, while patients with cirrhosis only showed an increase in VWF propeptide and FVIII levels. High molecular weight VWF multimers and VWF-dependent platelet adhesion increased in patients with hemophilia one hour after DDAVP administration, but did not change in the patients with cirrhosis. Levels of ADAMTS13 were unaffected in both patient groups after DDAVP.

Conclusion: The lack of relevant effects of DDAVP of laboratory indices of primary hemostasis in patients with cirrhosis is in line with previous clinical study results in these patients.
INTRODUCTION

Cirrhosis is associated with multiple changes in the hemostatic system including thrombocytopenia and platelet function defects, decreased circulating levels of pro- and anticoagulant factors, increased levels of von Willebrand factor (VWF) and factor VIII (FVIII) and decreased levels of fibrinolytic proteins (1). Cirrhosis has long been considered as a bleeding disorder, but it has become generally accepted that the hemostatic changes in cirrhosis may result in both bleeding and thrombotic complications (2). Nevertheless, reversal of the coagulopathy of these patients is frequently required in case of bleeding episodes, or as prophylaxis before invasive procedures.

Administration of 1-deamino-8-D-arginine vasopressin (DDAVP) has been shown to correct the skin bleeding time in patients with cirrhosis (3-5). Not much is known on the mechanism by which DDAVP would shorten the bleeding time in cirrhosis. The efficacy of DDAVP in patients with mild hemophilia A or type 1 Von Willebrands disease has been ascribed to an elevation of circulating levels of VWF and factor FVIII (6). However, since VWF and FVIII levels in cirrhosis are already substantially elevated (7, 8), it is unclear whether a further elevation in levels would exert any relevant prohemostatic effect. It has been demonstrated previously that VWF and FVIII levels increase in patients with cirrhosis after an intravenous, but not after a subcutaneous injection of DDAVP (3-5). Clinical data available from controlled studies indicate a lack of efficacy in patients with bleeding varices and in patients undergoing liver transplantation (9, 10).

The multimeric composition of VWF is controlled by a VWF-cleaving protease, ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13). A complete deficiency of this protein results in diffuse microthrombosis as a result of spontaneous platelet clumping by ultra-large VWF multimers, a disease referred to as thrombotic thrombocytopenic purpura (11). Infusion of DDAVP has been shown to result in a transient decrease of ADAMTS13 plasma levels, which may reflect consumption of ADAMTS13 in the process of active proteolysis of the DDAVP-induced release of ultra-large VWF molecules (12).

We investigated the potential pro-hemostatic effects of DDAVP in patient with cirrhosis to provide a rationale for the use of DDAVP as a pro-hemostatic agent in such patients.

METHODS

Patient group

Ten adult patients with stable cirrhosis (Child-Pugh score B or C) and ten adult patients with hemophilia A were included in this study between October 2011 and August 2013.
Exclusion criteria for both groups were known malignancies, active infection, renal failure, congenital hemostatic disorders (other than hemophilia A), recent transfusion of blood products, and the use of vitamin-K antagonist therapy. A small questionnaire was used to collect demographic information.

Written informed consent was obtained from every subject participating in this study. The study was approved by the local Medical Ethics Committee from the University Medical Center Groningen. Study procedures were in accordance with the Helsinki Declaration of 1975. Hemophilia patients were recruited from the Erasmus University Medical Center in Rotterdam, and the study was approved by the local ethical review board.

**Intervention**

All participating patients received a single bolus dose of DDAVP (0.3 μg/kg) through an intravenous catheter.

**Plasma samples**

Prior to, and 1, 3, 6, and 24 hours after DDAVP administration blood samples were drawn by vena-puncture and collected into vacuum tubes containing 3.8% trisodium citrate as an anticoagulant, at a blood to anticoagulant ratio of 9:1. Platelet poor plasma was prepared by double centrifugation at 2000g and 10.000g respectively for 10 min. Plasma was snap-frozen in liquid nitrogen and stored at -80 °C until use.

**VWF and ADAMTS13 assays**

Plasma levels of VWF were determined with an in-house enzyme-linked immunosorbent assay (ELISA) using commercially available polyclonal antibodies (DAKO, Glostrup, Denmark). VWF propeptide levels were determined using a commercially available ELISA from GTI Diagnostics (Aachen, Germany) according to the manufacturer’s protocol.

ADAMTS13 activity was measured in plasma which was pretreated with bilirubin oxidase (10U/mL; Sigma-Aldrich, Zwijndrecht, The Netherlands) to avoid interference of bilirubin with the assay (13). ADAMTS13 activity was assessed using the FRETS-VWF73 assay (Peptanova, Sandhausen, Germany) based on method described by Kokame et al (13). The antigen levels of VWF and the activity of ADAMTS13 in pooled normal plasma were set at 100%, and values obtained in test plasmas were expressed as a percentage of pooled normal plasma.

**VWF multimers**

VWF multimer analysis was performed by sodium dodecyl sulfate agarose gel electrophoresis followed by western blotting. The blots were incubated with rabbit anti-VWF antibody (DAKO) and goat anti-rabbit IRDye 800 CW (LI-COR Biosciences, Lincoln, NE). The first five bands were considered as low-molecular weight multimers, whereas
other bands were considered as high molecular weight (HMW) multimers. The blots were scanned by the Odyssey Imager (Westburg, Leusden, The Netherlands) and were quantified by morphometric analysis using the ImageScope software package (Aperio, Vista, CA). After shading correction and interactive thresholding, the selected positive pixels were measured. The positive area was the sum of the area of positive pixels of low-molecular weight and HMW bands. Data was expressed as the percentage of HMW multimers per total VWF multimers, which equals the percentage of positive pixels in the HMW band area per total positive pixel area.

**Factor VIII**
Levels of FVIII were measured on an automated coagulation analyzer (ACL 300 TOP) with reagents and protocols from the manufacturer (Hemosil (R) SynthASil and FVIII depleted plasma; Instrumentation Laboratory, Breda, the Netherlands).

**Platelet Adhesion Assay**
The ability of VWF to support platelet adhesion was studied under flow conditions in a reconstituted blood model. Red blood cells and platelets were isolated from whole blood of healthy volunteers who had blood group O as described previously (14). Cells were mixed with patient plasma or plasma from healthy volunteers to obtain reconstituted blood with a hematocrit of 40% and a platelet count of 250,000/μL. VWF-dependent platelet adhesion in reconstituted blood samples was assessed using a cone and plate viscometer (Diamed Impact R, Turnhout, Belgium). Uncoated Diamed wells were perfused at shear rate of 1,800/second for 2 minutes according to the instructions of the manufacturer. Platelet adhesion was quantified using May-Grünwald staining followed by software-assisted morphometric analysis using the Diamed apparatus and software delivered by the manufacturer.

**Statistical analyses**
Data are presented as medians with interquartile range (IQR) or as numbers with percentages. The one-way ANOVA with Dunnett’s post hoc test was used to compare levels of VWF, VWF propeptide, FVIII, and ADAMTS13 at the various time points after DDAVP administration to the baseline values. Correlations between VWF levels or differences in VWF level after DDAVP and the Child-Pugh score were determined by Pearson’s correlation coefficient. The paired t-test was used to analyze differences in VWF multimer release and VWF-dependent platelet adhesion between baseline and 1 hour after DDAVP administration. A p-value <0.05 was considered statistically significant. Analyses were performed using GraphPad Prism (San Diego, USA) and the statistical software package SPSS 15.0 (SPSS Inc., Chicago, IL).
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RESULTS

Patient characteristics

Patient characteristics are shown in Table 1. Patients with cirrhosis had higher levels of VWF, FVIII and VWF propeptide at baseline compared to patients with mild hemophilia A. Baseline levels of VWF in patients with cirrhosis increased with the severity of disease as assessed by the Child-Pugh scores ($r=0.85$, $p=0.002$). Baseline levels of ADAMTS13 were comparable in both groups. VWF propeptide /antigen ratio was strongly increased at baseline in patients with cirrhosis.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Hemophilia A n=10</th>
<th>Cirrhosis n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46 (40-55)</td>
<td>55 (47-58)</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>10/0</td>
<td>8/2</td>
</tr>
<tr>
<td>BMI</td>
<td>26 (23-32)</td>
<td>26 (24-35)</td>
</tr>
<tr>
<td>Child-Pugh Classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child B</td>
<td>N/A</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Child C</td>
<td>N/A</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Etiology of liver disease (number of patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biliary cirrhosis</td>
<td>N/A</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Alcoholic cirrhosis</td>
<td>N/A</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>Haemochromatosis</td>
<td>N/A</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Alcohol + NASH and NASH</td>
<td>N/A</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>NASH</td>
<td>N/A</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Laboratory values (at baseline)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>not determined</td>
<td>7.2 (6.4-8.3)</td>
</tr>
<tr>
<td>Platelets (10^9/L)</td>
<td>242.0 (168.8-260.3)</td>
<td>95.5 (58.5-148.5)</td>
</tr>
<tr>
<td>INR</td>
<td>not determined</td>
<td>1.4 (1.3-1.8)</td>
</tr>
<tr>
<td>Creatinine (umol/L)</td>
<td>not determined</td>
<td>66.5 (53.8-88.3)</td>
</tr>
<tr>
<td>Bilirubin (umol/L)</td>
<td>not determined</td>
<td>89.5 (53.0-126.0)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>not determined</td>
<td>31 (26.5-33.5)</td>
</tr>
<tr>
<td>VWF (%)</td>
<td>114 (94.5-164.3)</td>
<td>521 (238-643.8)</td>
</tr>
<tr>
<td>ADAMTS13 (%)</td>
<td>76.6 (57.3-87.9)</td>
<td>93.9 (59.2-130.4)</td>
</tr>
<tr>
<td>VWF propeptide (%) (%) (U/dl)</td>
<td>108.5 (97.3-121.3)</td>
<td>284 (236.5-392.8)</td>
</tr>
<tr>
<td>FVIII (%)</td>
<td>20 (12.3-38)</td>
<td>152.5 (117.3-193.8)</td>
</tr>
</tbody>
</table>

Data are presented as medians with IQR, as ratio, or as numbers with percentages.
N/A=not applicable; BMI= body mass index; NASH= non-alcoholic steatotic hepatitis; INR= international normalized ratio; VWF= Von Willebrand factor; ADAMTS13= a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; FVIII= factor VIII.
**VWF, VWF propeptide, FVIII, and ADAMTS13 after DDAVP administration**

Following administration of DDAVP, patients with hemophilia showed a significant increase in levels of VWF after 1 hour, followed by a steady decrease over time (Figure 1). In patients with cirrhosis, there was no significant change in levels of VWF following DDAVP administration. However, in some patients with cirrhosis, VWF levels slightly increased. The difference in VWF levels between baseline and 1 hour after DDAVP administration was inversely correlated with Child-Pugh scores ($r=0.72$, $p=0.019$). In other words, only in those patients with a low Child-Pugh score, a slight increase in VWF levels was detected.

Despite the absence of an increase in VWF plasma levels in patients with cirrhosis, levels of VWF propeptide did increase significantly 1 hour after DDAVP administration, although the relative increase in VWF propeptide following DDAVP in patients with hemophilia was much more pronounced (Figure 1).

Both patients with hemophilia and patients with cirrhosis showed an increase in levels of FVIII 1 hour after DDAVP administration, but the increase in the patients with cirrhosis did not reach statistical significance (Figure 2).

![Figure 1](image.png)

*Figure 1.* VWF antigen (A,B) and VWF propeptide (C,D) levels in patients with hemophilia (A,C) or cirrhosis (B,D) at baseline (t0), and 1, 3, 6, and 24 hours (t1, t3, t6, t24) after DDAVP administration. Bars indicate medians, error bars indicate IQR. * indicates $p<0.05$; ** indicates $p<0.01$; *** indicates $p<0.001$, all versus baseline.
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Patients with hemophilia

Patients with cirrhosis

Figure 2. FVIII (A, B), and ADAMTS13 (C, D) levels in patients with hemophilia (A, C) or cirrhosis (B, D) at baseline (t0), and 1, 3, 6, and 24 hours (t1, t3, t6, t24) after DDAVP administration. Bars indicate medians, error bars indicate IQR. * indicates p<0.05; versus baseline.

Figure 3. A. Proportion of HMW-VWF multimers in plasma from patients with hemophilia (left) or patients with cirrhosis (right) taken at baseline (t0) and 1 hour after DDAVP administration (t1). B, C. Capacity of plasma from patients with hemophilia (left) or patients with cirrhosis (right) at baseline (t0) and 1 hour after DDAVP administration (t1) to support platelet adhesion (B) and aggregation (C) under conditions of flow. Bars indicate medians, error bars indicate IQR.
ADAMTS13 activity did not change over time following DDAVP administration in both patients with cirrhosis and patients with hemophilia (Figure 2).

**VWF multimer pattern and VWF-dependent platelet adhesion following administration of DDAVP**

One hour following DDAVP administration, the proportion of high molecular weight VWF multimers increased significantly in patients with hemophilia but not in the patients with cirrhosis (Figure 3A). In line with the increase in the proportion of high molecular weight VWF multimers, VWF-dependent platelet adhesion and aggregation under conditions of flow substantially increased 1 hour after DDAVP administration in patients with hemophilia. Although there was a slight increase in platelet adhesion and aggregation in the patients with cirrhosis, this difference did not reach statistical significance (Figure 3B and C).

**DISCUSSION**

The combined results of our investigation show that administration of a single standardized dose of DDAVP to patients with cirrhosis resulted in minor changes in indices of primary hemostasis compared to changes observed following DDAVP administration to patients with mild hemophilia A. The hemostatic effect of DDAVP is assumed to be dependent on elevation of circulating levels of VWF and FVIII. Whereas VWF and FVIII substantially increased in patients with mild hemophilia A, the effects in patients with cirrhosis were marginal. Although these results and published clinical studies (9, 10) suggest a lack of hemostatic effect of DDAVP in patients with cirrhosis, we did observe a slight, although not statistically significant, improvement of the capacity of patient plasma to support platelet adhesion in a flow-based model. The latter results are consistent with the improvement in skin bleeding time following administration of DDAVP (3-5).

Although there are a number of studies showing a lack of clinical effect of DDAVP in patients with cirrhosis, a recent randomized controlled study suggested that DDAVP is as effective as transfusion of blood products in preventing blood loss in patients (15). However, a drawback of this study was the absence of a non-treated control group, which makes it impossible to determine whether DDAVP and blood products are equally effective in preventing blood loss, or that neither intervention is effective. The latter possibility is plausible in view of the recently changed insights in the hemostatic management of patients with cirrhosis (1, 16).

Two previous studies in which DDAVP was administered intravenously to patients with cirrhosis showed significant elevations of VWF plasma levels (3,4), whereas one
study employing subcutaneous DDAVP reported no increase in VWF plasma levels (5). Although we cannot fully explain the discrepancy between the studies, we speculate that only in those patients with relatively mild disease and consequently moderately elevated VWF levels, administration of DDAVP can result in a slight elevation of VWF plasma levels. Indeed, in the two studies in which DDAVP administration did result in significant elevations of VWF plasma levels, baseline VWF levels were much lower than in the present study as well as the study in which subcutaneous DDAVP was used.

The lack of VWF increase in patients with more advanced cirrhosis might be related to continuous endothelial cell stimulation or dysfunction as described in cirrhotic patients (17, 18). Continuous activation of endothelial cells in cirrhosis might lead to exhaustion of VWF in the endothelial cells rendering DDAVP stimulation ineffective. It has to be noted that the level of endothelial cell activation (and thus the baseline VWF level) may differ according to the etiology of disease, but to our knowledge this has not been studied in detail. As our small patient cohort consisted primarily of patients with alcoholic liver disease, and as our cohort did not include patients with hepatitis-associated cirrhosis, it is unclear whether our results are valid for cirrhosis of all etiologies.

Another explanation for the lack of increase in plasma VWF levels might be that DDAVP-mediated released VWF in cirrhotic patients is instantly consumed. Such a consumptive process may either occur systemically, or within the diseased liver. Thrombi within the cirrhotic liver have been demonstrated previously, providing support for intrahepatic consumption (19). Alternatively, DDAVP-induced VWF may remain attached to the activated endothelial cells which are known to have the capacity to bind VWF (20). Consumption or endothelial attachment of DDAVP-released VWF would explain the increase in VWF propeptide levels in patients with cirrhosis, which is released simultaneously with VWF from endothelial cells (21).

In contrast to a previous study in healthy volunteers and patients with type 1 von Willebrand’s disease (12), we did not find a decrease in ADAMTS13 plasma levels following DDAVP administration. We have no explanation for this discrepancy, but do note that there is no reason to assume that ADAMTS13 is consumed, inhibited, or cleared following VWF proteolysis. The normal ADAMTS13 levels in patients with cirrhosis are at variance, with some papers describing decreased levels of ADAMTS13 in cirrhosis (22, 23). Nevertheless, also normal to elevated levels of ADAMTS13 in cirrhosis have been described in humans (14) and experimental animal models (24), which may be related to the fact that the stellate cell is the primary site of ADAMTS13 biosynthesis (25).

We studied the effects of DDAVP administration in plasma-based assays of primary hemostasis, as the DDAVP-induced increases in VWF and FVIII are thought to be important determinants of its hemostatic effect. However, DDAVP also appears to have direct stimulatory effects on platelets (26,27), and future studies will be required to assess
effects of DDAVP on platelets from patients with cirrhosis which is of particular interest as cirrhosis may be associated with platelet function defects (28).

Our combined results suggest DDAVP to have minimal hemostatic effects in patients with cirrhosis. DDAVP may be effective in patients with mild disease and relatively low baseline VWF plasma levels, but clinical studies with relevant clinical endpoints will be required to ascertain this.
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REFERENCES


DDAVP does not improve primary hemostasis in patients with cirrhosis


