DESMOpressin and platelets antagonize the in vitro platelet dysfunction induced by GPIIb/IIIa inhibitors and aspirin

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Abstract

While bleeding is the most frequent adverse event encountered in patients receiving glycoprotein (GP) IIb/IIIa inhibitors, currently no recommendations exist how to treat such patients. The present study tested the hypothesis that infusion of desmopressin (DDAVP) reverses the in vitro platelet dysfunction induced by GPIIb/IIIa inhibitors (+L-aspirin). Study group 1 (ten healthy volunteers) received a DDAVP infusion to establish dose response curves for the in vitro inhibition of platelet function by eptifibatide, abciximab and tirofibran together with L-aspirin before/after DDAVP. Further, in a randomized, double-blind, placebo controlled, cross-over study (group 2) volunteers received L-aspirin and a standard eptifibatide infusion. Thereafter, DDAVP or a physiologic saline infusion were given over 30 min. In group 1 all GPIIb/IIIa inhibitors prolonged collagen/-epinephrine & -adenosine diphosphate closure times (CEPI-CT & CADP-CT), measured with the platelet function analyzer 100 (PFA-100). DDAVP caused a shift in the concentration response curves to the right of all three GPIIb/IIIa inhibitors. In group 2 DDAVP accelerated the normalization of CADP-CT & CEPI-CT after stop of eptifibatide infusion with a maximum effect at 1.5-2h. In contrast, CEPI-CT remained above normal in the placebo group for >4h. In conclusion, DDAVP accelerates normalization of the in vitro platelet dysfunction induced by GPIIb/IIIa inhibitors (+L-aspirin).

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Introduction

Platelet aggregation plays a central role in the formation of hemostatic plugs and arterial thrombi. Endothelial damage and plaque rupture result in the exposure of substances like collagen that promote platelet adhesion, activation, aggregation, and subsequent thrombus formation. GPIIb/IIIa is a platelet membrane receptor for fibrinogen, von Willebrand factor (VWF), vitronectin, and fibronectin, but the binding of fibrinogen and VWF is most critical for platelet aggregation. These polyvalent adhesive proteins cross-link GPIIb/IIIa (αIIb/βIII integrin) on the surface of activated platelets to cause platelet aggregation 1.

Since several large randomized trials have shown the therapeutic efficacy of GPIIb/IIIa blockers in patients with acute coronary syndromes, they have become a mainstay for the treatment of acute coronary syndromes 2. As expected from their mechanism of action, bleeding is the principle adverse effect from GPIIb/IIIa inhibitors. Yet, there are no antidotes to counteract GPIIb/IIIa blockers when treated patients are bleeding, although case reports or retrospective studies describe successful management of patients with platelet transfusions, who received the GPIIb/IIIa inhibitor abciximab and underwent emergency bypass grafting 3-6.

It has been demonstrated that the vasopressin agonist desmopressin (DDAVP) improves primary hemostasis and is a first line therapy for mild to moderate VWF-deficiency or hemophilia 7. In addition, DDAVP has been shown to improve congenital or acquired platelet dysfunction such as found in uremia and liver cirrhosis, and drug-induced bleeding related to heparin, hirudin, dextran, aspirin.
or ticlopidine. DDAVP is known to increase plasma levels of VWF and Factor VIII. An increased presence of high multimeric forms of VWF after DDAVP has been related to its haemostatic effectiveness and to its release from endothelial cells. It has recently been demonstrated that the release of VWF from endothelial storage pools improves primary hemostasis measured with the platelet function analyzer (PFA-100). Thus, DDAVP infusion increases VWF:RCo activity in patients with type 1 von Willebrand disease and prolonged collagen/epinephrine & -adenosine diphosphate closure times (CEPI-CT & CADP-CT) were normalized in all of these patients after DDAVP infusion. The in vivo VWF-release effects the normalization of CT-values in vitro.

We tested the hypothesis that DDAVP infusion would reverse the in vitro platelet dysfunction induced by GPIIb/IIIa inhibitors based due to its well-known pro-haemostatic properties.
Methods

Study Design
The study was approved by the Ethics Committee of Vienna University. Written informed consent was obtained from all subjects. Part 1 of this study was an open, prospective trial in ten healthy volunteers (group 1), who received DDAVP (Octostim®, Ferring AG, Vienna, Austria). Dose-response curves were established for the in vitro platelet inhibition by eptifibatide (Integrilin®, AESCA, Traiskirchen, Austria), tirofiban (Aggrastat®, Merck Sharp & Dome, Vienna, Austria) or abciximab (Reopro®, Eli Lilly, Vienna, Austria) together with a fixed concentration of acetylsalicylic acid (Aspisol®, L-ASA, Bayer, Vienna, Austria) before and after the increase in VWF-activity induced by DDAVP. Part 2 was a randomized, placebo controlled cross-over study trial in ten other healthy volunteers (group 2), who had not been part of group 1, receiving DDAVP and placebo. The randomisation was performed by randomisation lists in blocks of two and subjects crossed over to the alternative treatment after a wash out period of 8 days.

Subjects
Twenty healthy, non smoking, male volunteers (19 to 40 years, BMI 24 ±3 kg/m²) were included into the study. Physical health was defined as the absence of disease detectable by medical history, physical examination, routine laboratory and virologic parameters as described elsewhere13. Exclusion criteria were history of drug allergy or recent (within 3 weeks) intake of any drugs.
Treatment

In group 1, DDAVP (0.3µg/kg in 50mL physiologic saline) was infused over 30 minutes (min) after the baseline blood sample had been drawn. Further venous samples were taken with minimal stasis (whenever possible no tourniquet was applied for blood sampling) 15 and 30 min, and 1, 2, 4, 6 and 8 hours (h) post dosing from an antecubital vein contralateral to the infusion.

To mimic a clinical situation in group 2, all subjects received acetylsalicylic acid (250mg iv bolus) as well as a standard eptifibatide infusion (180µg/kg bolus followed by a continuous infusion of 2µg/kg/min for 2h) on both study days, after the baseline blood sample (−2h) had been drawn. For this purpose, we chose eptifibatide because abciximab more frequently induces severe thrombocytopenia in patients and even in healthy subjects14; 15. As would be done in case of bleeding, the eptifibatide infusion was then stopped. Thereafter, 50mL of a DDAVP (0.4µg/kg) or a physiologic saline (placebo) infusion was given over 30 min. A second cannula on the arm (contralateral to the infusion) permitted further blood samplings at 0.5, 1, 1.5, 2, 4, 6 and 8h post dosing. Before and after blood sampling the venous line was rinsed with 5 mL of physiologic saline to avoid plugs in the cannula without the need for anticoagulants, discarding 10 mL of blood before any blood sampling.

All volunteers (group 1 and 2) reported 24h after the drug administration for a final blood sample (venipuncture with 21-gauge butterfly needle).
Blood collection

Blood was drawn into 4mL siliconized glass tubes (Vacutainer Greiner bio-one, Kremsmünster, Austria) containing 3.8% sodium citrate for determination of the \textit{in vitro} aspirin effect on collagen/-epinephrine closure times (CEPI-CT)\textsuperscript{12; 16} in group 1. However, in citrated blood samples the inhibitory effect of eptifibatide is overestimated\textsuperscript{17} and the CEPI-CT is prolonged by aspirin\textsuperscript{16}. Thus, for determination of the effects of GPIIb/IIIa inhibitors, collagen/-adenosine diphosphate closure times (CADP-CT), CEPI-CT and platelet aggregation units, specimen were drawn into 4ml native glass tubes (Vacutainer Greiner bio-one) containing 400µl lepirudin (final conc.: 25µg/mL lepirudin whole blood) (Refludan\textsuperscript{®}, a kind gift from Hoechst, Vienna, Austria) in group 2.

Blood counts

Platelet counts were performed with a cell counter (Sysmex Counter, Milton Keynes, UK).

Von Willebrand Factor Ristocetin Cofactor Activity (VWF:RCo) Levels

VWR:Co was assayed by turbidometry using a commercial kit from Behring (Marburg, Germany) which consists of lyophilized platelets and ristocetin.

Platelet function analyzer 100 (PFA-100)

In this study we used the PFA-100 (Dade Behring, Vienna, Austria) which measures \textit{in vitro} platelet plug formation under high shear stress\textsuperscript{18}. The instrument records the time necessary for the occlusion of the aperture, defined as the closure time (CT)\textsuperscript{19}. Aspirin prolongs the CEPI-CT activated blood,
whereas the CADP-CT is minimally affected\textsuperscript{16}. All measurements were done within one hour of blood sampling.

**Aspirin and GPIIb/IIIa inhibitors: in vitro incubation experiments**

Whole blood (980µl) was incubated with 20µl of acetylsalicylic acid (final concentration 50mg/L) for 3 min at room temperature to compare the influence of acetylsalicylic acid in *citrated* and *lepirudinised anticoagulated whole blood* on the CADP-CT and CEPI-CT. The selected concentrations of GPIIb/IIIa inhibitors we used were based on previous reports\textsuperscript{20-25} and on our prestudy experiments (data not shown). For the determination of the CADP-CT, *lepirudin anticoagulated whole blood* (980µl) was incubated with acetylsalicylic acid (10µl; final conc. 50mg/L) and additionally either eptifibatide (10µl; final conc.: 200, 400, 800 and 1600ng/mL), abciximab (10µL; final conc.: 1000, 1500 and 3000ng/mL) or tirofiban (10µL; final conc.: 20, 40 and 80ng/mL) for 3 min at room temperature. The time period for the *in vitro* incubation experiments were selected on a previous report\textsuperscript{16} and on our prestudy experiments (data not shown).

**Rapid platelet function assay**

In group 2 the aggregation of fibrinogen-coated beads was measured with the rapid platelet function assay (Accumetrics, Genozyme Virotech, Rüsselsheim, Germany)\textsuperscript{26}. The rapid platelet function assay provides information on platelet function that mirrors platelet aggregation and reflects GPIIb/IIIa receptor blockade\textsuperscript{27}. Thrombin receptor activating peptide in the cartridge activates platelets, which bind and agglutinate fibrinogen-coated beads with a consequent
increase in light transmittance. The light absorbance of the sample is measured as a function of time, and the rate of agglutination is quantified as platelet aggregation units. To calculate the percent inhibition for the subjects who received the GPIIb/IIIa inhibitor eptifibatide, a baseline platelet aggregation unit value was obtained prior to administration of the GPIIb/IIIa inhibitor drug. *Lepirudin anticoagulated whole blood* was analyzed according to the manufacturer’s instructions at room temperature within one hour of sample collection.

**Bleeding time**

The bleeding time was determined before any treatment (–2h) and immediately after the DDAVP infusion using a commercially available template method (Simplate® II R, Eppelheim, Germany). A blood pressure cuff was put on the upper arm (pressure 40 mmHg) and a standardized horizontal incision (5 mm long and 1 mm deep) was created on the volar part of the forearm. Thereafter, blood was wicked from the cut with filter paper until the bleeding had stopped (maximal observation period, 30 min), at which point the time was recorded (normal range: 3 to 10 min).

**Statistical Methods**

For descriptive statistics all data is expressed as means ± standard deviation (SD) for description of results in text or ranges unless otherwise stated. We used a two-way ANCOVA for analysis of treatment effects first (treatment = independent factor, subject = covariant, outcome variable = dependent factor in a repeated measures design). All subsequent statistical comparisons were done
by the Friedmann ANOVA and the Wilcoxon signed ranks test for post hoc comparisons. A two-tailed p-value of less than 0.05 was considered significant.
Results

Basal closure times
In good agreement with previous reports\textsuperscript{18}, basal CEPI-CT of \textit{citrated blood} averaged 133s (range: 88-176s), excluding one outlayer in Fig. 1, and mean basal CADP-CT was 109s (range: 78-133s). In \textit{lepirudinised blood} closure times were reduced as compared to citrate: the basal CEPI-CT averaged 120s (range: 73-155s) and the basal CADP-CT was 87s (range: 57-117s).

Aspirin effect \textit{in vitro}: Comparison between citrated and lepirudinised blood
L-aspirin, added \textit{in vitro} to whole blood (50mg/L) (Fig. 1, A) or infused into volunteers (250 mg) (Fig. 1, B) prolonged the CEPI-CT to >300s \textit{in vitro} in \textit{citrated blood} (p=0.005). In contrast, in \textit{lepirudinised blood}, the CEPI-CT was much less affected (from 120±21s to 151±43s, p<0.05) (Fig. 1, C) and the CADP-CT was not prolonged \textit{in vitro} (87±13s before aspirin, 83±12s after aspirin, p>0.05) (Fig. 1, D) after infusion of 250 mg L-aspirin.
Fig. 1 Effect of L-aspirin on *in vitro* platelet plug formation in citrated or lepirudin anticoagulated blood. L-aspirin was added *in vitro* to whole blood (50mg/L) (A) or infused into volunteers (250mg) (B) for determination of collagen/epinephrine closure time (CEPI-CT) in *citrated blood*. For the determination of CEPI & collagen/adenosine (CADP)-CT in *lepirudinised blood* L-aspirin was infused into volunteers (250mg), respectively (C, D). L-aspirin prolonged the CEPI-CT (n=10) in *citrated blood* (A, B) whereas in *lepirudinised blood* the CEPI-CT (n=8) was less influenced (C) and the CADP-CT (n=10) was not prolonged *in vitro* (D).
**VWF:RCo**

In **group 1**, DDAVP increased plasma VWF:RCo levels 2.8-fold from 132±60 U/mL to 377±65 U/mL at 1h (p=0.005, vs baseline) (Fig. 2). Likewise, in **group 2**, VWF:RCo increased 2.8-fold from 108±18 U/dL to 302±26 U/dL 30 min after infusion of DDAVP and reached a maximum at 1.5h (397±50 U/dL; p=0.005, vs baseline and between treatment). Placebo did not affect VWF:RCo-levels at any time (range: 110 to 140 U/dL).

**Platelet counts**

Platelet counts were not affected by treatment with DDAVP in **group 1**. The maximum change in any individual was 11%. In **group 2**, platelet counts were not affected by any drug: compared to baseline, the maximum change in platelet counts in any individual was 4% after eptifibatide infusion. The maximum change in the DDAVP period was 7% and in the placebo period 10 %.

**DDAVP, Aspirin and GPIIb/IIIa inhibitors: Relationship and concentration effect**

DDAVP infusion shortened both CEPI-CT & CADP-CT with a maximum effect at 30 min: CEPI-CT decreased from 133±27s to 71±12s and CADP-CT decreased from 86±18s to 55±7s (p<0.01) (Fig. 2, **group 1**). More importantly, after adding L-aspirin *in vitro* to the blood samples, DDAVP decreased the L-aspirin induced inhibition of platelet function in a biphasic manner: (i) complete normalization was seen immediately after DDAVP infusion: CEPI-CT was reduced from 292±28s to 145±61s (p<0.01) at 30 min. (Fig. 2). (ii) the aspirin effect was significantly mitigated up to 2h after the start of DDAVP infusion in all subjects.
Further, adding eptifibatide, tirofiban or abciximab \textit{in vitro} to whole blood, all three GPIIb/IIIa inhibitors concentration-dependently prolonged both CEPI & CADP–CT \textit{in vitro} \((p<0.01, n=10)\) (Fig. 3, group 1). After DDAVP infusion higher concentrations of all three antagonists were necessary to inhibit platelet plug formation (Fig. 3). Hence, DDAVP inhibited the effect of all three GPIIb/IIIa inhibitors at submaximal concentrations and caused a shift in their concentration-response curves to the right.

Infusion of eptifibatide prolonged both CEPI & CADP-CT in \textit{lepirudinised blood}: CEPI-CT increased from 120±21s to >300s \((p=0.005)\) and CADP-CT increased from 87±13s to 262±62s \((p<0.001)\) 2h after start of eptifibatide infusion (Fig. 4, group 2). DDAVP shortened both CEPI-CT & CADP-CT after discontinuation of eptifibatide infusion with a maximum effect at 1.5-2h \((p<0.05 \text{ and } p<0.01, \text{ between treatments})\) (Fig. 4). Compared to the values immediately after eptifibatide infusion, CADP-CT decreased from 276±17s (0h) to 66±5s 1.5h after start of DDAVP infusion \((p<0.01)\), and from 246±22s to 113±6s in the placebo period at 1.5h \((p<0.01)\). In parallel, DDAVP decreased CEPI-CT from >300s to 134±35s at 1.5h \((p<0.05)\), whereas CEPI-CT decreased from >300s to 240±30s in the placebo period at 1.5h \((p>0.05)\) (Fig. 4).
Fig. 2 Desmopressin infusion improves the in vitro platelet dysfunction induced by L-aspirin. Ten healthy volunteers received DDAVP (0.3µg/kg) over 30 min. L-Aspirin (50mg/L) (open symbol) was added in vitro to blood samples before and after DDAVP infusion. Horizontal dashed lines show the upper and lower limits of normal CEPI-CT & CADP-CT values. Data is presented as mean ±SEM. *p<0.05, **p<0.01 and ***p=0.005 vs baseline. DDAVP increased von
Willebrand Ristocetin Cofactor activity (vWF:RCo) levels (top) and thereby shortened both CEPI-CT (citrated blood) (middle) and CADP-CT (lepirudinised blood) (bottom). DDAVP decreased the aspirin-induced inhibition of platelet function (middle: open symbol) with a normalization at 30 minutes and a persistent response for 8 hours.
Fig. 3 Desmopressin (DDAVP) shifts concentration/response curves of GP IIb/IIIa antagonists to the right. GP IIb/IIa inhibitors were added to whole blood in vitro before and after DDAVP infusion. Eptifibatide (top), tirofiban (middle) and abciximab (bottom) concentration-dependently inhibited platelet function, as measured by the PFA-100. Horizontal dashed lines show the upper limit of...
normal CT values. The horizontal solid lines show the maximal measurable CT. Data is presented as mean ± SEM. *p<0.05, **p<0.01 (before DDAVP vs after DDAVP). DDAVP-infusion caused a shift in the concentration response curves of all three GP IIb/IIIa inhibitors to the right in lepirudinised blood (n=10).
Fig. 4 Desmopressin accelerates reversal of *in vitro* platelet dysfunction after discontinuation of eptifibatide infusion as compared to placebo. Ten healthy volunteers received on both study days L-aspirin (250mg iv bolus) and a standard eptifibatide infusion for 2h. After stop of eptifibatide infusion DDAVP or
placebo were infused. Eptifibatide prolonged both CEPI & CADP–CT in lepirudinised blood. Data is presented as mean ±SEM. *p<0.05, **p<0.01 between groups; †p<0.05, ‡p<0.01 compared to the values after eptifibatide infusion. DDAVP significantly accelerated normalization of both CEPI-CT & CADP-CT after stop of eptifibatide infusion with a maximum effect at 1.5-2h while the initial CADP-CT values (0-1h) were not affected. In contrast, CEPI-CT remained above normal in the placebo group for > 4h, possibly reflecting an aspirin-like defect.
Rapid platelet function assay

The mean level of platelet inhibition was >92% in all subjects immediately after the stop of the eptifibatide infusion (Tab. 1). A satisfactory level of inhibition (>80%) was measured until 1.5h after the end of the eptifibatide infusion. A low level of inhibition of GPIIb/IIIa receptors (<80%) was obtained 2h after the stop of eptifibatide infusion irrespective of concomitant treatment with placebo or DDAVP (Tab. 1).

Tab. 1 Effect of eptifibatide on platelet glycoprotein GPIIb/IIIa receptor blockade measured with the Rapid platelet function assay.

<table>
<thead>
<tr>
<th>Hours after eptifibatide infusion</th>
<th>Placebo period</th>
<th>Desmopressin period</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95 ±1 (93-100)</td>
<td>94 ±1 (92-97)</td>
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<tr>
<td>0,5</td>
<td>90 ±2 (80-97)</td>
<td>90 ±2 (83-95)</td>
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<tr>
<td>1,5</td>
<td>82 ±1 (80-83)</td>
<td>85 ±1 (82-90)</td>
</tr>
<tr>
<td>2</td>
<td>77 ±2 (73-80)</td>
<td>78 ±3 (72-88)</td>
</tr>
</tbody>
</table>

Values are percent, mean ±SEM and ranges.
Bleeding time

Eptifibatide increased the bleeding time from 7 min (range: 4-11 min) to 22 min (range: 13-30 min) in the placebo period and from 6 min (range: 3-8 min) to 19 min (range: 8-30 min) in the DDAVP period (p=0.005 vs baseline). No significant shortening of bleeding time was observed immediately after the end of DDAVP infusion (p>0.05).

Correlation between methods

Under basal conditions CADP-CT correlated with CEPI-CT (r= 0.85, p=0.014) and both with the bleeding time (r=0.66-0.68, p<0.05). CADP-CT showed a good correlation with the bleeding time (r=0.83, p=0.003) 30 min after eptifibatide infusion. In contrast, the rapid platelet function assay showed only a trendwise correlation with the bleeding time (r=0.63, p>0.05) and did not correlate with CEPI or CADP-CT. Interestingly, 1.5h after start of DDAVP infusion, both CEPI or CADP-CT were essentially corrected to normal, while rapid platelet function assay -blockade was still 85%.

Safety aspects

We believe this is the first study where healthy volunteers received currently accepted standard doses of both eptifibatide and L-aspirin. All clinical adverse effects observed were attributed to concomitant infusion of DDAVP (Tab. 2). None of the 20 volunteers (group 1 and 2) experienced any severe or serious adverse events. All symptoms stopped spontaneously on the same day in all subjects.
Tab. 2 Adverse events after drug infusion

<table>
<thead>
<tr>
<th>SYMPTOMS</th>
<th>TREATMENT</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
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<td></td>
<td>DDAVP</td>
<td>Eptifibatide &amp; L-aspirin</td>
<td>Eptifibatide, L-aspirin &amp; DDAVP</td>
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<td>n=2</td>
<td>n= 4</td>
</tr>
<tr>
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<td>n= 3 (syst) n= 5 (diast)</td>
<td>n= 5 (syst) n= 7 (diast)</td>
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<tr>
<td>facial flush</td>
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<td>n= 2</td>
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<td>nausea</td>
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<td>n=0</td>
<td>n= 0</td>
</tr>
<tr>
<td>thrombocytopenia (&lt;150 x10^9/L)</td>
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</tr>
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</table>
Discussion

This is the first study to demonstrate that DDAVP accelerates normalization of the *in vitro* platelet dysfunction induced by GPIIb/IIIa inhibitors (+L-aspirin) in healthy subjects. For repeat assessment of primary hemostasis, we used the PFA-100\textsuperscript{18}. As the inhibitory effect of eptifibatide is overestimated in citrated blood samples\textsuperscript{17}, lepirudin anticoagulated blood was used for determination of all measurements.

First, group 1 showed the expected 3-fold increase in VWF:RCo levels after DDAVP infusion; DDAVP thereby shortened both CEPI-CT and CADP-CT (Fig. 2). Secondly, DDAVP normalized the aspirin-induced *in vitro* platelet dysfunction immediately at the end of infusion and significantly ameliorated the aspirin defect of platelet function up to 2h (Fig. 2). Thirdly, DDAVP shifted concentration response curves for all three GPIIb/IIIa inhibitors to the right *in vitro* (Fig. 3). However, DDAVP did not antagonize the effect at maximal concentrations of GPIIb/IIIa inhibitors. Thus, DDAVP antagonizes the effect induced by GPIIb/IIIa inhibitors, particularly at submaximal concentrations *in vitro*.

These results of group 1 were further confirmed in group 2: First, DDAVP infusion did not shorten the 3-fold prolongation in bleeding time compared to placebo 0.5 h after stop of eptifibatide infusion. This is in good agreement with the lack of DDAVP effect on closure times (PFA-100) immediately after the end of DDAVP infusion (Fig. 4). At the time when the bleeding time was measured (0.5h) CADP-CT was not significantly different between groups, although CEPI-CT was trendwise shorter in the DDAVP period (possible due to normalization of
the aspirin-component of platelet defect). CADP-CT averaged 160s, which is similar to patients, after coronary artery bypass graft, who excessively bleed, but who are responsive to platelet transfusions. Secondly, DDAVP accelerated the normalization of CADP-CT & CEPI-CT after stop of eptifibatide infusion with a maximum effect at 1.5-2h. In contrast, CEPI-CT remained above normal in the placebo group for >4h, possibly reflecting an aspirin-like defect (Fig. 4). Hence, DDAVP appears to have no measurable effect at maximal concentrations of GpIIb/IIIa inhibitors in vivo confirming the ex vivo experiments (Fig. 3). Compared to placebo, DDAVP accelerates normalization of platelet dysfunction induced by eptifibatide with a maximum effect at 1.5-2h, when plasma eptifibatide levels are reduced to submaximal levels (Fig. 4). The receptor occupancy of 80-85% (measured by the rapid platelet function assay, Tab. 1) was still in the therapeutic range at that time Summarizing, DDAVP can help to normalize the in vitro platelet dysfunction quickly even at therapeutic levels of GPIIb/IIIa blockade and is expected to shorten bleeding episodes.

The CADP-CT correlated with the bleeding time under basal conditions and after infusion of eptifibatide. After eptifibatide infusion this correlation reached $r^2=0.69$. There was a relatively good agreement ($r=0.83$) between the in vivo bleeding time and ex vivo assessment of platelet function by PFA-100. The value of the bleeding time in the prediction of bleeding disorders, however, is controversially discussed. In contrast, the rapid platelet function assay showed only a trendwise correlation with CADP-CT and the bleeding time. The lack of a perfect correlation between these methods is likely attributable to differences between in vivo and in vitro systems with or without high shear rates.
The current study has some limitations: First, bleeding complications during administration of GPIIb/IIIa inhibitors are not very common and for this reason, a randomized study in bleeding patients is not easily conducted. The relative contribution of GPIIb/IIIa inhibitors to bleeding may be difficult to differentiate from heparin-induced bleeding in the individual case. Hence, we have to assume that GPIIb/IIIa inhibitors could enhance bleeding in patients who receive these agents along with aspirin and heparin. However, DDAVP responsiveness in patients with acute coronary syndromes has not been formerly studied; whether such patients will respond to DDAVP in the same manner as normal individuals is not clear. Secondly, eptifibatide was given as a single bolus in this trial, as our study was designed before the ESPRIT-study (ESPRIT: Enhanced Suppression of the Platelet Receptor glycoprotein IIb/IIIa using Integrilin Therapy [in patients undergoing PCI]) had been published, in which eptifibatide bolus was given twice. However, the double bolus primarily affects plasma concentrations early after start of infusion, and we have none-the-less achieved clinically relevant receptor occupancy rates of 90-95%. Thirdly, and importantly the PFA-100 and rapid platelet function assay test are artificial measures of primary hemostasis and do not necessarily reflect in vivo hemostasis. Finally, there may be concerns to use DDAVP in patients after PCI because of potential adverse effects, such as fluid retention or thrombotic events due to DDAVP as indicated by case reports. We assume that the former problem can be managed by close observation of patients and fluid restriction, and that restoration of normal flow and normal shear rates after PCI will decrease the likelihood of acute thrombosis which might be facilitated by VWF-release.
Despite these limitations, the following clinical recommendations may be given: DDAVP should be used whenever bleeding is suspected to stem from GP IIb/IIa inhibitors. As long as there are no clinical trials in bleeding patients, we recommend the following course of action based on our “proof of concept study”: (i) stop the GPIIb/IIa infusion (ii) obtain a platelet count and aPTT, (iii) if possible, measure the degree of platelet inhibition with a rapid bedside test, (iv) administer a DDAVP infusion and/or (v) transfuse platelet concentrates in case of major or life threatening bleeding or urgent need for normalization of platelet function in case of surgery.

In conclusion, DDAVP accelerates normalization of \textit{in vitro} platelet dysfunction induced by GPIIb/IIa inhibitors as soon as levels of GPIIb/IIa inhibitors are reduced to levels of borderline effectiveness.
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Desmopressin and platelets antagonize the in vitro platelet dysfunction induced by GPIIb/IIIa inhibitors and aspirin

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