Shear-Induced Platelet Aggregation Is Potentiated by Desmopressin and Inhibited by Ticlopidine

Marco Cattaneo, Rossana Lombardi, Donato Bettega, Anna Lecchi, and Pier Mannuccio Mannucci

Shear-induced platelet aggregation is important in physiological hemostasis and in the pathogenesis of arterial thrombosis. It requires extracellular Ca\textsuperscript{2+}, platelet membrane glycoproteins Ib/IX and IIb/IIIa, von Willebrand factor (vWF), and ADP. We studied the effects of desmopressin (DDAVP), which increases plasma vWF levels and shortens the bleeding time, and of ticlopidine, which inhibits platelet responses to ADP, on shear-induced platelet aggregation. Eleven healthy volunteers were given oral ticlopidine (250 mg b.i.d.) for 7 days. The same subjects were infused intravenously with DDAVP (0.3 µg/kg body wt) before the first and after the last doses of ticlopidine. The degree of platelet aggregation induced by shear stress at 25, 50, 75, and 100 dyne/cm\textsuperscript{2} in a cone-and-plate viscometer, plasma vWF levels, and the bleeding time were measured before and after each DDAVP infusion. Plasma vWF levels and the extent of shear-induced platelet aggregation increased after DDAVP and were correlated. Ticlopidine partially inhibited shear-induced platelet aggregation both before and after DDAVP infusion. The bleeding time, prolonged by ticlopidine, was shortened by DDAVP. Potentiation by DDAVP of shear-induced platelet aggregation may be one mechanism by which the drug shortens the prolonged bleeding time. Since shear-induced platelet aggregation can cause thrombotic occlusions in stenotic arterial vessels, our findings may explain the therapeutic efficacy of ticlopidine in arterial thrombosis. (Arteriosclerosis and Thrombosis 1993;13:393–397)

KEY WORDS • shear stress • platelet aggregation • desmopressin • ticlopidine • von Willebrand factor • ADP • bleeding time

Platelet aggregation, which plays an important role in physiological hemostasis\textsuperscript{1} and in the pathogenesis of arterial thrombosis,\textsuperscript{2} has an absolute requirement for the platelet membrane glycoprotein complex IIb/IIIa\textsuperscript{3,4} (GP IIb/IIIa), which binds adhesive proteins such as fibrinogen,\textsuperscript{5,6} von Willebrand factor (vWF),\textsuperscript{7,8} fibronectin,\textsuperscript{9,10} and vitronectin\textsuperscript{4,11} after platelet activation. Studies performed with the light-transmission aggregometer\textsuperscript{12} have shown that fibrinogen is the adhesive protein that preferentially binds to stimulated platelets and supports platelet aggregation.\textsuperscript{13–17} However, if platelet aggregation is studied under controlled conditions of high shear stress, it is vWF rather than fibrinogen that supports platelet aggregation.\textsuperscript{18–22} At high shear, platelets may aggregate independently of exogenous platelet agonists, with aggregation triggered by the interaction of vWF with the platelet membrane GP Ib/IX complex, followed by the binding of vWF to the receptor for adhesive proteins exposed on the platelet GP IIb/IIIa complex.\textsuperscript{18–22} The “supranormal” multimers of vWF that are present in endothelial cells and platelets are more efficient than the largest vWF multimers present in normal plasma in supporting shear-induced platelet aggregation.\textsuperscript{18–21}

Since the rheological conditions that can be found in the normal microcirculation or at sites of severe arterial stenosis are characterized by high shear,\textsuperscript{23} pharmacological modulation of platelet aggregation at high shear may be clinically relevant. Potentiation might improve primary hemostasis but heighten the risk of thrombus formation in stenotic arteries, whereas inhibition might impair primary hemostasis but reduce the risk of arterial thrombosis. Inhibitors of shear-induced platelet aggregation are substances that interfere with the binding of vWF to GP Ib/IX or GP IIb/IIIa complexes\textsuperscript{18–22,24} and prostaglandins that increase platelet cyclic adenosine monophosphate.\textsuperscript{25} Partial inhibition can be accomplished with ADP scavengers, suggesting that the ADP released from platelets contributes to the formation of shear-induced platelet aggregates.\textsuperscript{21,26} In contrast, drugs that interfere with the platelet cyclooxygenase pathway, such as acetylsalicylic acid, have no inhibitory effects.\textsuperscript{21,27}

In this study, the effects on shear-induced platelet aggregation of two commonly used drugs that affect the hemostatic system, ticlopidine and desmopressin (DDAVP), were tested. Ticlopidine is an antiaggregating agent with antithrombotic effects,\textsuperscript{28} which selectively...
inhibits platelet responses to ADP and prolongs the bleeding time. DDAVP, a synthetic analogue of vasopressin, increases the plasma levels of vWF and factor VIII and shortens the bleeding times of normal individuals and patients with congenital and acquired defects of primary hemostasis.

**Methods**

**Materials**

[14C]Serotonin (5-hydroxytryptamine-3'-[14C]creatinine sulfate, 57.5 mCi/nmol) was obtained from Amersham International (Buckinghamshire, UK). ADP, the endoperoxide analogue 9-epoxymethanoprostaglandin F2a, U46619, collagen, and Triton X-100 were purchased from Sigma Chemical Co., St. Louis, Mo. All chemicals were of reagent grade or better. DDAVP (Minirin DDAVP) was from Valeas, Milano, Italy, and ticlopidine (Ticlid) from Sanofi-Winthrop, Milano, Italy.

**Treatment Protocol**

Eleven healthy volunteers (six women, five men; aged 27-36 years) were given oral ticlopidine (250 mg b.i.d.) for 7 days. DDAVP (0.3 μg/kg body wt) was infused intravenously over 30 minutes before the first and after the last dose of ticlopidine.

Before and 5 minutes after each infusion of DDAVP, the bleeding time was measured (Symplate II, Organon Teknika, Milan, Italy), and blood was taken from an antecubital vein and anticoagulated with 12.9 mmol/L trisodium citrate.

**Shear-Induced Platelet Aggregation**

Blood samples were centrifuged at 150g at room temperature for 10 minutes to obtain platelet-rich plasma (PRP). The platelet count in the PRP was adjusted to 3 x 10^9/L with autologous platelet-poor plasma (PPP), obtained by centrifugation of blood at 1,200g for 15 minutes.

Duplicate PRP samples (350 μL) were exposed to controlled shear stress levels (25, 50, 75, and 100 dyne/cm2) in a stainless steel cone-and-plate viscometer (Controlled Stress Rheometer, Carri-med, Dorking, UK) at 37°C for 45 seconds. The cone diameter was 6 cm and its angle 0.21°; the distance between the cone and the plate was adjusted with an electronic sensor. After being subjected to shear stress, 25 μL of PRP was added to 100 μL of 2.5% paraformaldehyde in phosphate-buffered saline, and the number of single platelets per microliter was counted under a phase-contrast light microscope. The appearance of platelet aggregates was accompanied by a decrease in the number of single platelets. Thus, the percent increase in platelet aggregation was directly related to the percent decrease in platelet count. Results were expressed as a percentage of single platelet counts of PRP incubated in the cone-and-plate viscometer at 37°C for 45 seconds without shearing.

**Platelet Lysis**

To determine the extent of platelet lysis caused by shear forces, 150 μL of the PRP remaining on the plate of the viscometer was centrifuged in an Eppendorf microcentrifuge for 2 minutes to obtain PPP, in which the concentration of lactate dehydrogenase (LDH) was determined with a commercial kit (LDH opt, Boehringer, Mannheim, FRG). The results were expressed as percent of LDH concentration in 1% Triton X-100 lysates of PRP, after subtraction of the concentration present in the PPP of unsheared samples.

**Platelet Aggregation and Release Reaction Measured in the Light-Transmission Aggregometer**

Twenty milliliters of blood was collected in 12.9 mmol/L trisodium citrate on the first day of treatment (before the administration of ticlopidine), before and after the infusion of DDAVP. Platelet aggregation and release of [14C]serotonin induced by ADP (1 and 2 μmol/L), collagen (1 μg/mL), or U46619 (0.25 and 0.5 μmol/L) were measured in an Elvi Chronolog aggregometer (Milano, Italy), as previously described.

**Analysis of Plasma von Willebrand Factor**

Measurement of plasma vWF antigen (vWF:Ag) and of the ristocetin cofactor activity (vWF:RiCof) levels and the multimeric analysis of vWF were performed as previously described, using an electroimmunoassay (for vWF:Ag), an aggregometric assay with formalin-fixed platelets (for vWF:RiCof), and low-resolution,

**Figure 1.** Line plot showing effects of desmopressin (DDAVP, 0.3 μg/mL) and ticlopidine (250 mg b.i.d. for 7 days) on shear-induced platelet aggregation. Duplicate platelet-rich plasma samples were exposed to the indicated levels of shear stress in a cone-and-plate viscometer at 37°C for 45 seconds. At the end of shearing, platelet aggregation was calculated as the percent decrease in the number of single platelets (see "Methods" for details). *p<0.01 vs. before DDAVP; #p<0.01 vs. before ticlopidine.
TABLE 1. Effects of Intravenous DDAVP on Plasma von Willebrand Factor Levels of 11 Normal Volunteers

<table>
<thead>
<tr>
<th></th>
<th>Before DDAVP</th>
<th>After DDAVP</th>
<th>Before DDAVP</th>
<th>After DDAVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF:Ag (units/dL)</td>
<td>117±17</td>
<td>236±67*</td>
<td>110±47</td>
<td>224±69*</td>
</tr>
<tr>
<td>vWF:RiCof (units/dL)</td>
<td>106±44</td>
<td>228±16*</td>
<td>103±12</td>
<td>220±17*</td>
</tr>
</tbody>
</table>

DDAVP, desmopressin; vWF:Ag, von Willebrand factor antigen; vWF:RiCof, ristocetin cofactor activity. Values are mean±SEM. *p<0.001 vs. before DDAVP.

Sodium dodecyl sulfate–agarose gel electrophoresis (for vWF multimers).

Statistical Analysis
Results are expressed as mean±SEM. Differences among groups were analyzed using one-way analysis of variance, followed by the Student-Newman-Keuls test when appropriate.

Results
Shear-Induced Platelet Aggregation
Exposure of PRP to increasing controlled shear stresses for 45 seconds caused a progressive reduction in the number of single platelets per microliter (Figure 1), which was associated with the appearance of platelet aggregates. In all experiments, the percentage of platelet lysis, calculated as percent increase in plasma LDH concentrations, was always less than 5% and, therefore, the reduction in platelet count after shearing can be attributed mostly to the formation of platelet aggregates. Platelet aggregation did not occur at a shear stress of 25 dyne/cm², was modest at 50 dyne/cm², and progressively increased to about 40% at a shear stress of 100 dyne/cm².

The infusion of DDAVP caused a doubling of plasma vWF:Ag and vWF:RiCof levels (Table 1) and the appearance of supranormal vWF multimers (Figure 2). After DDAVP, shear-induced platelet aggregation increased (Figure 1): it was already detectable (approximately 20%) after exposure of PRP to a shear stress level of 50 dyne/cm² and increased to about 60% at a shear stress of 100 dyne/cm². A shear stress of 75 dyne/cm² after DDAVP induced the same degree of platelet aggregation induced by 100 dyne/cm² before DDAVP.

Ticlopidine partially inhibited platelet aggregation induced by shear stress (Figure 1). The inhibition was statistically significant at levels of shear stress of 75 and 100 dyne/cm² (p<0.01). The aggregation of ticlopidine-treated platelets after the infusion of DDAVP was comparable to that of untreated platelets before the infusion of DDAVP (Figure 1). Ticlopidine had no effects on either the increases in plasma vWF:Ag and vWF:RiCof levels (Table 1) or the appearance of vWF with supranormal multimers (Figure 2) caused by DDAVP.

Platelet aggregation induced by a shear stress of 100 dyne/cm² under basal conditions and after DDAVP administration was positively correlated with the plasma levels of vWF:Ag (r=0.50, p<0.001).

Bleeding Time
DDAVP significantly shortened the bleeding times of the 11 healthy volunteers at both baseline and after the prolongation induced by oral ticlopidine (Table 2).

Effects of Desmopressin on Platelet Aggregation and Release of [14C]Serotonin Induced by Platelet Agonists
The administration of DDAVP did not significantly affect the extent of platelet aggregation (Table 3) nor the release of [14C]serotonin (not shown) measured in the light-transmission aggregometer after stimulation of PRP with ADP, collagen, or U46619, indicating that the reactivity of platelets to exogenous agonists was not affected by DDAVP.

Discussion
The results of this study indicate that the degree of shear-induced platelet aggregation is correlated with plasma vWF levels and is potentiated by the infusion of DDAVP, which increases plasma vWF levels by inducing the release of vWF with supranormal multimers from endothelial cells. These ex vivo findings are consistent with the demonstration that vWF plays a key role in shear-induced platelet aggregation\(^{14,22}\) and that sup-
pranormal vWF multimers derived from human endothelial cells grown in vitro are more effective than the largest multimers normally present in plasma. The increase in plasma vWF levels induced by DDAVP explains the normalization of the bleeding time in patients with defects of vWF. However, DDAVP shortens the bleeding times not only of patients with von Willebrand disease, but also of normal subjects and of patients with congenital or acquired defects of primary hemostasis who have normal plasma vWF levels. Although mechanisms independent of released vWF are certainly operative, it has been suggested that the effect of the drug might also be mediated by the increase in plasma vWF that it induces. This study supports this hypothesis, since it shows that DDAVP potentiates the aggregation of normal and abnormal (ticlopidine-treated) platelets that occurs at levels of shear that can be found in the microcirculation and, therefore, are relevant to bleeding time measurements. The increased aggregation occurring at high shear stress after DDAVP is probably the result only of the induced increase in plasma vWF concentrations and the appearance of supranormal vWF multimers, since platelet reactivity to aggregating stimuli is not affected by DDAVP, as shown by our light-transmission aggregometer studies.

Recent reports have cautioned against the use of DDAVP for individuals with evidence of coronary and/or cerebral atherosclerosis, since it may precipitate acute myocardial infarction or ischemic stroke. This study elucidates the pathophysiology of such severe clinical complications. Shear stress increases abruptly to shear-induced platelet aggregation can be modulated by DDAVP, as shown by our light-transmission aggregometer studies. In conclusion, this is the first ex vivo study showing that shear-induced platelet aggregation can be modulated in normal subjects by drugs that interfere with the hemostatic system. Modulation of shear-induced platelet aggregation may be useful both for the treatment of patients with hemorrhagic disorders and for the management of patients at risk for arterial thrombosis.

References


TABLE 2. Effects of Intravenous DDAVP and Oral Ticlopidine on the Bleeding Times of 11 Normal Volunteers

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Before DDAVP</th>
<th>After DDAVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP (2 µmol/L)</td>
<td>38±5</td>
<td>30±4</td>
</tr>
<tr>
<td>U46619 (1 µmol/L)</td>
<td>31±5</td>
<td>22±7</td>
</tr>
<tr>
<td>Collagen (2 µg/mL)</td>
<td>55±3</td>
<td>47±4</td>
</tr>
</tbody>
</table>

DDAVP, desmopressin; U46619, 9-epoxymethanoprostaglandin F3α (endoperoxide analogue). Values are mean±SEM.

TABLE 3. Effects of Intravenous DDAVP on Agonist-Induced Platelet Aggregation of 10 Normal Volunteers

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Percent light transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before DDAVP</td>
<td>After DDAVP</td>
</tr>
<tr>
<td>ADP (2 µmol/L)</td>
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</tr>
<tr>
<td>U46619 (1 µmol/L)</td>
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</table>

DDAVP, desmopressin; U46619, 9-epoxymethanoprostaglandin F3α (endoperoxide analogue). Values are mean±SEM.
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