Platelet Thrombus Formation on Collagen Type I
A Model of Deep Vessel Injury
Influence of Blood Rheology, von Willebrand Factor, and Blood Coagulation

Lina Badimon, Juan Jose Badimon, Vincent T. Turitto, Shankar Vallabhajosula, and Valentin Fuster

Collagen type I is a major component of atherosclerotic vessel wall that is exposed on deep vessel injury, such as in balloon angioplasty or plaque rupture. Collagen type I from pig Achilles tendon was mounted in a tubular perfusion chamber placed within an extracorporeal circuit (carotid artery to jugular vein). The material was exposed to blood from normal pigs (n=13), severe homozygous von Willebrand factor (vWF)-deficient pigs (vWF<3%) (n=6), and heterozygous vWF-deficient pigs (vWF=24%) (n=2). Thrombus formation was measured by autologous 111In-platelet labeling and by ultrastructural morphology. Heparinized and native blood from these pigs was perfused over the substrate for 3 and 5 minutes at local shear rates from 212 to 3,380/sec. On collagen type I exposed to nonanticoagulated blood, for all exposure times studied, thrombus formation in the absence of vWF was significantly reduced at high shear rate typical of stenotic areas but not at low shear rate typical of unobstructed medium-size arteries. A similar inhibition in thrombus formation due to vWF deficiency was observed in both heparinized and native blood; however, thrombus formation was significantly more reduced (p<0.05) in the presence of heparin, presumably due to the lack of stability of the accumulated platelets in the absence of fibrin formation. Intermediate levels of vWF, as in heterozygous von Willebrand's disease (vWD), support platelet deposition to extents not significantly different from normal conditions. Therefore, on collagen type I, both the activation of blood coagulation proteins and the presence of vWF contribute significantly to the platelet-platelet interactions necessary for thrombus formation. The effect of vWF occurs primarily at high shear conditions typical intravascularly of flow at the apex of advanced stenotic lesions; thus, these findings may suggest that the absence of vWF may be protective against the development of acute thrombosis in these regions. (Circulation 1988;78:1431-1442)

Thrombosis is a process that involves various blood cells and proteins, the nature of the blood flow, and surface characteristics. In vivo, all blood-surface interactions are dynamic in nature; that is, they occur in the presence of flowing blood. Therefore, blood elements are exposed to fluid transport rates and local shear forces that differ substantially from those that occur in stagnant or tube reaction systems. Various studies have demonstrated that flow conditions can affect both qualitatively and quantitatively the deposition of cells and proteins on surfaces.

There is increasing evidence that the acute clinical manifestations of coronary atherosclerosis are due to plaque fissuring or rupture. Although there has been considerable controversy regarding the causal role of coronary thrombosis in acute myocardial infarction, angiographic studies and the successful application of thrombolytic therapy make it certain that most cases of acute myocardial infarction are caused by coronary thrombosis. The rheological changes in the coronary circulation due to coronary arteriosclerosis are probably factors contributing to the acute coronary syndromes. At the stenotic sites of atherosclerotic vessels, local
shear rate conditions may be considerably increased compared with conditions in a fully patent vessel. For example, it is well known that reduction in tubular cross-sectional area results in a substantial increase in the local shear rate (cubic for the ideal case of well-developed flow) when the local flow rate is unchanged. In addition, plaque rupture can result in exposure of the deeper layers of the vessel wall and of thrombogenic elements of the plaque itself. Collagen type I is a major component of the normal vessel wall and of atherosclerotic plaque; therefore, we have used native type I collagen fibrils as a model of vessel injury.

The von Willebrand factor (vWF), a multimeric glycoprotein of high molecular weight, has been shown to influence platelet deposition on subendothelial surfaces and to lead to a bleeding diathesis in patients with a severe abnormality. The absence of vWF leads to a shear-dependent defect in platelet attachment to subendothelium; more recently, a role for vWF in platelet-platelet interaction has also been proposed.

The aim of the present study has been to investigate thrombus formation on collagen type I under well-characterized flow conditions. We are particularly concerned with the extent to which vWF is involved in the stabilization and control of the thrombus growth on this substrate to investigate the potential for this factor to modulate the acute thrombosis involved in the complications of coronary atherosclerosis. Heparinized or nonanticoagulated blood from normal pigs and from pigs homozygous or heterozygous for von Willebrand’s disease (vWD) was used in the present studies. Blood-surface interaction was studied for a broad range of shear rates ranging from those typical of medium-size unobstructed arteries to those in stenotic vessels and the microcirculation in a cylindrical perfusion chamber placed within an extracorporeal perfusion system.

We found that the absence of vWF in both heparinized and nonanticoagulated blood significantly reduces platelet thrombus formation at high wall shear rates (stenotic or microcirculatory flow) but not at shear rates of unobstructed medium-size arteries. Furthermore, the absence of vWF produces a considerably greater reduction in platelet deposition compared with anticoagulation with heparin. These findings may suggest a role for vWF in the thrombotic complications associated with stenotic cardiovascular disease and that under high shear conditions the acute thrombotic response may be more sensitive to manipulation of vWF than of procoagulant factors.

Materials and Methods

Experimental Model

Our experimental model was the swine. All procedures performed in this study were approved by the appropriate institutional guidelines and followed the American Heart Association Guidelines for animal research.

Swine with inherited vWD were from the colony kept at Mayo Clinic by Dr. E.J. Bowie (Mayo Institute Hills Farm, Mayo Foundation). The homozygote pig has the observed impairment of primary hemostasis and the other hemostatic abnormalities noted in the severe form of the disease in humans such as an autosomal recessive transmitted bleeding tendency, a long bleeding time, reduced retention of platelets in a column filled with glass beads, reduced levels of factor VIII, very low levels (<3% of normal) of vWF, and a lack of Ristocetin cofactor (RC o) in plasma. No vWF is present in the platelet granules. The homozygote pigs have no bleeding tendency and intermediate levels of factor VIII, vWF, and RC o in plasma. All pigs were housed at the Animal Research Center pig facilities of our institution. They were individually caged in a light-, temperature-, and humidity-controlled environment, with ad libitum access to water and controlled food intake (3% wt/wt of body weight) (Purina Laboratories).

Determination of Hemostatic and Lipid Parameters

Bleeding time, factor VIII, vWF, and vWF:RCo were measured by assay methods previously described. Table I shows the hemostatic parameters of the normal and vWD pigs used in this study. Platelet number and hematocrit were evaluated by our previously described methods (Table 2).

Platelet aggregation was measured in whole blood and in platelet-rich plasma as previously reported in swine. The agonists used for whole blood impedance aggregometry were adenosine 5’-diphosphate (ADP) (final concentration, 5 and 10 μM) (Sigma Chemical, St. Louis, Missouri) and collagen (final concentration, 1 and 2 mg/ml) (Chrono-log Corp, Haverton, Pennsylvania); the extent of aggrega-

<table>
<thead>
<tr>
<th>Table 1. Hemostatic and Lipid Parameters</th>
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<tr>
<td></td>
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<tr>
<td>Normal</td>
</tr>
<tr>
<td>Homozygous vWD</td>
</tr>
<tr>
<td>Heterozygous vWD*</td>
</tr>
<tr>
<td>Factor VIII:C (%)</td>
</tr>
<tr>
<td>vWF (%)</td>
</tr>
<tr>
<td>vWF:RCo (%)</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

vWD, von Willebrand disease; vWF, von Willebrand factor; RCo, Ristocetin cofactor.

*Average of two animals; no variation about the mean is given.
tion was determined from the maximum height of response in ohms (10 chart paper units=5 Ω) and the rate of aggregation from the slope at the steepest part of the curve (Ω/min). ADP (5 and 10 μM) and collagen (1, 2, and 5 μg) were used as agonists in optical aggregometry, and the extent of aggregation was calculated from the maximum change in light transmission (%). Platelet aggregation was not significantly different in the three genotypes when whole blood or platelet-rich plasma was challenged with the above agonists.

**Perfusion Chamber**

We have used an original perfusion chamber that mimics the cylindrical shape of the blood vessels. In this chamber, a circumferential portion of the tubular flow channel is replaced by the test substrate material (length, 2.5 cm), which becomes directly exposed to the blood. Two chambers of different internal diameters (1.0 and 2.0 mm) provide a broad range of wall shear rates on the substrate with moderate changes in average blood flow rate (Table 3).

The selected flow rates were 10 and 20 ml/min in the small and large chamber. These flows gave theoretically calculated average blood velocities from 5.3 to 42.3 cm/sec and local shear rates from 212 to 3,380/sec. These shear rates correspond to values ranging from those of large, healthy (unobstructed) arteries (106–500/sec) to values typical of the developing flow regions of branched vessels or the stenotic areas of the atherosclerotic vessels as well as to normal values in the microcirculation (1,680 and 3,380/sec). At these shear rates, blood can be considered a Newtonian fluid with constant viscosity. Shear conditions at the vessel wall were calculated from the expression for shear rate given for a Newtonian fluid in tube flow.

**Test Surface**

Collagen type I strips were obtained from pig Achilles tendon. The tendons were dissected, cleaned of surrounding connective tissue, frozen (liquid N2), and stored at −70°C. On the day of use, they were thawed in cold (4°C) Tris buffer. Strips 3 cm in length and varying from 0.7 to 0.8 cm in width were separated from placement in the perfusion chamber. Collagen type I has been shown to be a highly reactive surface with respect to platelets in previous studies performed in flow systems and perfusion chambers.

We have characterized the collagen biochemically and ultrastructurally. Normal pig Achilles tendon was homogenized and solubilized by pepsin digestion. Collagens were precipitated, dialyzed, and pelleted by ultracentrifugation as previously described. The resultant nonfibrillar collagen type I was characterized by sodium dodecyl sulfate-5% polyacrylamide gel electrophoresis. The typical configuration of collagen type I can be observed in Figure 1.

**Table 2. Blood and Platelet Values**

<table>
<thead>
<tr>
<th></th>
<th>Platelets (×10³/µl)</th>
<th>Mean platelet volume (µ³)</th>
<th>Hematocrit (%)</th>
<th>Red blood cells (×10³/µl)</th>
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</thead>
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<tr>
<td>Normal</td>
<td>430</td>
<td>7.1</td>
<td>32</td>
<td>7.0</td>
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<tr>
<td></td>
<td>50</td>
<td>0.2</td>
<td>1</td>
<td>0.2</td>
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<tr>
<td>Homozygous vWD*</td>
<td>393</td>
<td>6.8</td>
<td>33</td>
<td>6.8</td>
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<tr>
<td></td>
<td>18</td>
<td>0.1</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Heterozygous vWD*</td>
<td>332</td>
<td>7.8</td>
<td>32</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

vWD, von Willebrand disease.

*AVERAGE of two animals; no variation about the mean is given.

**Table 3. Flow Characteristics of Perfusion Chambers**

<table>
<thead>
<tr>
<th>Tubular diameter (d) (cm)</th>
<th>Blood flow rate (Q) (ml/min)</th>
<th>Blood average velocity (Vₐ) (cm/sec)</th>
<th>Blood wall shear rate (Yw) (l/sec)</th>
<th>Entrance length (Lₜ) (cm)</th>
<th>Reynolds number† (Re)</th>
</tr>
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<tbody>
<tr>
<td>0.2</td>
<td>5</td>
<td>2.6</td>
<td>105</td>
<td>0.05</td>
<td>15</td>
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<td>212</td>
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<td>30</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>10.6</td>
<td>425</td>
<td>0.18</td>
<td>60</td>
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<td></td>
<td>30</td>
<td>15.9</td>
<td>640</td>
<td>0.27</td>
<td>90</td>
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<td>40</td>
<td>21.1</td>
<td>850</td>
<td>0.36</td>
<td>120</td>
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<tr>
<td>0.1</td>
<td>5</td>
<td>10.4</td>
<td>840</td>
<td>0.05</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>21.2</td>
<td>1,680</td>
<td>0.09</td>
<td>60</td>
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<tr>
<td></td>
<td>20</td>
<td>42.4</td>
<td>3,360</td>
<td>0.18</td>
<td>120</td>
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<td></td>
<td>30</td>
<td>63.6</td>
<td>5,040</td>
<td>0.27</td>
<td>180</td>
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<tr>
<td></td>
<td>40</td>
<td>84.8</td>
<td>6,720</td>
<td>0.36</td>
<td>240</td>
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</table>

*Entrance length is the distance required to attain a fully developed velocity profile (Le=0.015 d Re).

†Reynolds numbers were calculated on the assumption that the blood viscosity (µ) was 3.5 centipoise and the blood density was 1.0 g/cm³ (Re=µ Vₐ² /ρµ).
Collagen type I bundles and vessel wall were processed for ultrastructural analysis. The specimens were fixed in a mixture of 3% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.4. For transmission electron microscopy, specimens were fixed in 1% OsO<sub>4</sub>, stained in block with 2% aqueous uranyl acetate, dehydrated with ethanol, and embedded in Epon 812. The specimens were examined under a transmission electron microscopy (Figure 2).

**Experimental Procedure**

The pigs were sedated with intramuscular Prom-Aze (2 ml) (acepromazine maleate, Fort Dodge Lab, Fort Dodge, Iowa)<sup>1,12</sup> followed by intravenous injection of sodium pentobarbital (Fort Dodge Lab). The carotid artery and contralateral jugular vein were catheterized by cutdown and an extracorporeal circuit (carotid artery–perfusion chamber–jugular vein) was established as previously described.<sup>1,12</sup> Two types of perfusion systems were used.

**Nonrecirculation experiments perfusing nonanticoagulated blood.** The carotid artery was connected to the input of the chamber, maintained at 37°C, and the output connected to a variable speed peristaltic pump (Model 7013, Master-flex) with interchangeable heads calibrated to obtain selected blood flow rates. Test specimens were mounted in the chamber and perfused with Vassar saline (pH 7.4) at 37°C for 60 seconds. Blood was then perfused over the substrate at a preselected flow rate and exposure time. At the end of the perfusion period, buffer was passed through the chamber for 30 seconds under identical flow conditions. Blood was discarded after exiting the chamber to prevent reinfusion of potentially activated blood.

**Recirculation experiments.** The animals were intravenously heparinized (300 units/kg) (Liquemin 10,000; Roche, Nutley, New Jersey). The cannulated carotid artery was connected to the input of the Plexiglas chamber. The output of the chamber was connected to the peristaltic pump. Blood that passed through the chamber was recirculated back into the animal by the contralateral jugular vein. The specimens were processed as before. They were perfused with Vassar saline solution at 37°C for 60 seconds. After the preperfusion period, blood at a preselected flow rate entered the chamber for various perfusion times. At the termination of the blood perfusion period, buffer was again passed for 30 seconds under identical flow conditions through the chamber and discarded. The changes from buffer to blood and vice versa were achieved by a three-way valve without the introduction of stasis in the chamber. The extracorporeal circulation produced no changes in hematocrit, platelet count, platelet lysis, or radiolabel uptake by various organ systems during the duration of the experiment, compared with experiments where blood was not recirculated.

**Radioactive Labeling of Platelets**

Approximately 24 hours before the perfusion experiment, autologous platelets were labeled with <sup>111</sup>In (tropolone) in plasma.<sup>1,35</sup> The final pellet of labeled platelets was resuspended in 4.5 ml platelet-poor plasma and injected into the animal after a low-speed centrifugation to remove any microaggregates. The labeling procedure required approximately 2 hours. An average indium plasma activity of 3.7±0.7% (mean±SEM) was measured just before injection of the platelet concentrate. The activity was 212±12 μCi (mean±SEM), and 3×10<sup>6±2×10<sup>5</sup></sub>/μl <sup>111</sup>In-labeled platelets (mean±SEM) were injected in a volume of 4.5 ml plasma.

The number of platelets deposited on each specimen was calculated from the platelet count and the
Figure 2. Microphotographs of longitudinal section of vessel wall (top panel) and Achilles tendon (bottom panel). Typical periodic band pattern with polarized repeat of collagen fibrils is seen in pig vessel wall (original magnification, ×20,000) and tendon (original magnification, ×9,000). Some transversally cut fibrils are also evident in both tissues.
Table 4. Native Blood Platelets Deposited (×10^6/cm^2) on Collagen Type I Bundles

<table>
<thead>
<tr>
<th>Shear rate (sec^-1)</th>
<th>Perfusion time (min)</th>
<th>Normal</th>
<th>Homozygous vWD</th>
<th>Heterozygous vWD</th>
</tr>
</thead>
<tbody>
<tr>
<td>212</td>
<td>3</td>
<td>47±10^1</td>
<td>74±16^1</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>...</td>
<td>81±16^1</td>
<td>...</td>
</tr>
<tr>
<td>1,690</td>
<td>3</td>
<td>256±20^3</td>
<td>68±13^3</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>...</td>
<td>109±15^5</td>
<td>478</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

vWD, von Willebrand’s disease.

Comparison of like numbers (e.g., 1-1 and 2-2) indicates that results indicated are statistically different for p<0.001.

*Results are given without deviation about the mean because these are averages of one perfusion in each of the two animals.

In-activity on the perfused area and in blood with a method previously described. Results were normalized by area of exposed surface.

Statistical Analysis

Results were statistically analyzed for the best bivariate data model fitting with repeated-measures analysis of variance and Duncan’s multiple range test, and the unpaired t test when applicable. Values are mean±SEM. The CLINFO and SAS computer system was used for all statistical analyses (supported by grant RR-71 from Division of Research Resources, National Institutes of Health).

Results

Platelet Deposition to Collagen Type I in Nonanticoagulated Blood

Local blood shear rate 212/sec. When nonanticoagulated blood was drawn at a rate of 10 ml/min from the carotid artery of normal swine and perfused over collagen fibrils for 3 minutes, platelets rapidly accumulated on the collagen to an average of 47×10^6 platelets/cm^2 (Table 4).

In perfusions performed with blood from homozygous or heterozygous vWD swine, platelet deposition at 3 minutes was not significantly different from normal blood perfusions (Table 4).

Local blood shear rate 1,690/sec. When normal blood was perfused at shear rate 1,690/sec for 3 minutes, mural white thrombus could be visibly observed at the end of the perfusion and an average deposition of 256×10^6 platelets/cm^2 was determined (Table 4). Similar results were obtained with blood from heterozygous vWD animals.

In contrast, homozygous vWD blood perfusion resulted in dramatically reduced levels (p<0.001) of platelet deposition on the collagen (68×10^6 platelets/cm^2) (Table 4).

Influence of Exposure Time on Shear Dependence of Platelet Deposition in Unanticoagulated Blood

Platelet deposition was determined for perfusion times ranging from 1 to 5 minutes to investigate whether the length of exposure influenced the difference in thrombosis on collagen fibrils observed between normal and vWF-deficient animals. In the present study, normal pigs were exposed for 1 and 3 minutes at the shear rates of 212 and 1,690/sec, and the total platelet deposit was normalized by the exposure time. Longer times were not used because thrombotic masses grew large enough to occlude the chamber. The heterozygous and homozygous animals were exposed for the longer times of 3 and 5 minutes and similarly normalized by time. The normalized results are shown in Figure 3. There was no apparent influence of exposure time on normalized platelet deposition in the various types of pigs, indicating that the increase in platelet deposition can be considered linear with exposure time within the range studied. The increase in platelet deposition with shear rate is significantly reduced in homozygous vWD pigs with respect to that observed in either normal or heterozygous vWD animals (p<0.05). No significant increase in platelet deposition with shear rate is observed in homozygous pigs as seen in Figure 3.

Figure 3. Bar chart of rate of platelet deposition on collagen (platelets×10^6/cm^2×sec^-1). Perfusion with nonanticoagulated blood from normal, homozygous von Willebrand's disease (vWD) and heterozygous vWD swine. Numbers in parentheses are number of experimental perfusions. The rate of platelet deposition at high shear is significantly greater than at low shear for normal and heterozygous vWD. In homozygous vWD, platelet deposition is independent of shear rate. Platelet deposition in normal and heterozygous vWD blood is similar. At high shear rates, the difference between both normal and heterozygous vWD and homozygous vWD is highly significant.
Platelet Deposition on Collagen in Heparinized Blood

In these experiments, the pigs were heparinized and the perfusion chamber placed within an extracorporeal circuit (carotid artery-jugular vein). Four different local blood shear rate conditions were studied. 

Local blood shear rate 212 and 424/sec. Blood was perfused over the collagen fibrils in a large diameter (0.2 cm) chamber at flow rates of 10 and 20 ml/min. At either 212 or 424/sec and perfusion times ranging from 1 to 5 minutes, no statistically significant difference in platelet deposition to collagen was observed among the three genotypes (values for 212/sec are shown in Figure 4), although reduced deposition was suggested in the homozygous vWD pigs. Values of platelet deposition ranged from 10 to $50 \times 10^6$ platelets/cm$^2$.

Local shear rate 1,690 and 3,380/sec. At these high local shear rate conditions, perfusions with normal blood yielded massive platelet deposits of up to $9 \times 10^6$ platelets/cm$^2$ (Figure 5); the deposit increased with both exposure time and shear rate. Mural white thrombus could be detected by visual observation of the perfused areas. Examination of the central area of the perfused surface with scanning electron microscope showed that mural platelet thrombus had already formed as early as 1 minute of perfusion (Figure 6). Platelet deposition at 1,690/sec was significantly increased compared with the deposition observed at 212 and 424/sec in normal blood. Deposition at 3,380/sec was also significantly increased over that at 1,690/sec ($p<0.05$).

In contrast, perfusion under similar conditions with blood from homozygous vWD swine resulted in only a slight increase in the number of platelets deposited compared with values obtained at low shear rates (Figure 5). When compared with values obtained with normal blood under identical experimental conditions, the reduction in platelet deposition in vWD pigs was highly significant ($p<0.001$).

Shear Rate Dependence of Platelet Deposition on Collagen in Heparinized Blood

In normal swine, the rate of platelet deposition did not show any increase as shear rate was increased from 212 to 424/sec. However, the deposition was significantly enhanced at 1,690/sec ($p<0.001$) and 3,380/sec ($p<0.001$) compared with the lower shear rates (Figure 7). In homozygous vWD, the rate of platelet deposition did not show dependence on local shear rate; the slight increase in platelet deposition at high shear rates was not statistically significant (Figure 7). In heterozygous vWD, the rate of platelet deposition increased with shear rate but remained somewhat lower than in normal animals.

Platelet deposition to collagen fibrils exposed to heparinized blood from normal (78 perfusions), homozygous vWD (36 perfusions), and heterozygous vWD (16 perfusions) swine from 1 to 5 minutes was normalized by exposure time for comparison of

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**Figure 4.** Plot of platelet deposition on collagen (platelets×10$^6$/cm$^2$). Heparinized blood of normal (●), homozygous von Willebrand's disease swine (●), and heterozygous von Willebrand's disease swine (○) was perfused at a low shear rate (212/sec) typical of unobstructed medium-size arteries. Numbers within parentheses are the total of experimental perfusions with blood from different animals. There is no significant difference in platelet deposition among the three genotypes at this shear condition.

**Figure 5.** Bar charts of platelet deposition on collagen (platelets×10$^6$/cm$^2$). Heparinized blood of normal (hatched bars), homozygous von Willebrand's disease swine (solid bars), and heterozygous von Willebrand's disease swine (open bars) swine was perfused over the substrate at shear rates typical of unobstructed medium-size arteries (212 and 424/sec) and of stenotic zones and microcirculation (1,690 and 3,380/sec). Note that the scale in ordinates is different from the other figures.
deposition rates. The rate of platelet deposition in homozygous vWD swine is significantly reduced from that of normal swine. However, the rate of platelet deposition in heterozygous vWD was not significantly different from normal.

Effect of Heparin on Platelet Accumulation on Collagen

In Table 5, the results of platelet deposition on collagen at 3 minutes of perfusion are depicted. The presence of heparin did not show any effect in normal animal platelet deposition at low shear rate; however, in the absence of vWF, the blockade of thrombin (or other procoagulant moities) significantly reduced platelet deposition. At high shear rate, in the absence of vWF and with heparin, the number of platelets deposited was the lowest of all. At high shear, the presence of vWF and the presence of activated coagulation enzymes resulted in a synergistic effect on platelet deposition compared with the effect of either of these variables.

Discussion

In the present study, we have demonstrated the stabilizing effect of vWF, a high molecular weight plasma glycoprotein, on thrombus growth on collagen type I, in both the presence and absence of heparin. In addition, we have shown that flow and specifically local shear rate conditions modulate attachment, growth, and stability of platelet masses on collagen (type I); that platelet attachment is directly related to local shear rates; and that the availability of coagulation enzymes has a significant stimulatory effect on platelet deposition rate on this substrate material.

Collagen Type I—A Thrombogenic Substrate

Ruptured atherosclerotic plaque with exposure of the plaque matrix to flowing blood has been shown to predispose to clinical thrombosis. Atherosclerotic lesions characteristically have an increased content of collagen, with type I being the predominant...
collagen.\textsuperscript{5-7,36} We have used collagen type I bundles from Achilles tendon as a model of the organized collagen fibers that may be exposed in severe arterial injury, although other components may also regulate the thrombotic response to injured vessel walls. This collagen has been biochemically characterized as type I (Figure 1) and appears similar ultrastructurally to vessel wall collagen (Figure 2). Our present studies show that the exposure of this thrombogenic component of the vessel wall leads to extensive platelet deposition and thrombosis on the surface, which eventually is able to block the chamber lumen. The stabilization of platelet masses observed on the collagen fibers contrasts with the gradual loss of accumulated platelets from subendothelium exposed under the same flow conditions.\textsuperscript{1,10}

The increased reactivity of collagen also correlates with our previous findings that mural thrombus formation is directly related to the extent of medial exposure in pigs undergoing carotid angioplasty.\textsuperscript{37} A major advantage of the isolated native collagen preparation used in the present study is that the deposition results are not influenced by substrate levels of vWF, tissue factor, or releasable eicosanoids as have been described in studies of vessel walls. In addition, because collagen fibrils in dense and areolar connective tissue and in arterial walls are associated with proteoglycans,\textsuperscript{38-42} the use of native collagen fibrils in our study renders a more physiological preparation than when purified collagens are used.\textsuperscript{43} In addition, the material can be used to further verify observations made with more complicated materials, such as the atherosclerotic vessel wall. The behavior of this substrate is influenced by the local shear conditions, the state of activation of the blood coagulation system, and the plasma content of vWF.

\textbf{Blood flow factors.} Blood flow forces directly influence the arrival and removal rates of platelets on the substrate. Thrombus formation is a highly dynamic process, and platelet deposits are a balance between the detachment and reattachment of cells.\textsuperscript{44} On the collagenous substrate perfused with normal blood, the rate and density of attached platelets increase significantly with local shear rate (Figure 7). These results are in contrast to deposits on subendothelium, which in general are several orders of magnitude less than on collagen and are dispersed with continuing exposure time. In the present study, high blood shear rates were unable to dislodge significant quantities of platelet thrombi, and deposits continued to increase with exposure time on the fibrillar collagen material. The rate and extent of deposition were found to be influenced by the blood concentration of vWF and the activation of the coagulation system as will be discussed below.

\textbf{Thrombus formation in nonanticoagulated blood.} We have found that at high local shear rate conditions (1,690/sec, Table 5), the platelet deposition rate was significantly higher in nonanticoagulated blood than in heparinized blood ($p<0.005$). However, at low local shear rates typical of medium-size unobstructed vessels (212/sec), there was little difference between heparinized and nonanticoagulated blood with respect to the deposition of platelets on the exposed collagen. In the present study, no measurement of the extent of fibrin formation has been attempted at the different shear conditions. It is likely that fibrin is much more extensive at the low shear conditions than at high shear conditions as has been reported on subendothelium,\textsuperscript{45} although such behavior has not been demonstrated with collagenous structures. In fact, no studies have been conducted in flowing blood systems to determine the mechanism by which fibrin may be activated by collagen type I. Crude preparations of collagen have been shown to initiate coagulation

![Figure 7](http://circ.ahajournals.org/)

\textbf{FIGURE 7. Plot of rate of platelet deposition on collagen (platelets x 10^4 x cm^-2 x min^-1).} Blood derived from heparinized swine (●), homozygous von Willebrand's disease swine (x), and heterozygous von Willebrand's disease swine (c) was perfused over the collagen bundles. (See explanation in text.)

<table>
<thead>
<tr>
<th>Table 5. Platelet Deposition on Collagen Type I</th>
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<tbody>
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<td>212/sec</td>
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</tr>
<tr>
<td>Unanticoagulated</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Homozygous vWD</td>
</tr>
</tbody>
</table>

\textsuperscript{vWD, von Willebrand disease.} Values are mean±SEM (x 10^6 platelets/cm^2). Perfusion time in the experiment was 3 minutes.
intrinsically through Factor XII, in contrast to the tissue factor-related activation pathway described for subendothelium. Clearly, studies conducted in this area will be important to the understanding of collagen as a stimulant of both platelet and coagulant activity. In any case, our results suggest that the level of procoagulant material and fibrin produced at low shear rates does not markedly influence the platelet deposition. That is, under these flow conditions, the predominant interaction is platelet-collagen association where the rate is determined primarily by the local shear conditions, whereas at high shear rate, the deposition, which is now both platelet-collagen and platelet-platelet in nature, becomes independent of flow factors and more dependent on stabilization by procoagulant materials generated by the blood-surface interaction.

The production of thrombin probably underlies the enhanced ability of platelets to deposit in native blood. However, heparin may have effects on other components of the blood in addition to anticoagulation. In a recent publication, we have demonstrated in vitro (absence of procoagulant moieties) that platelet deposition in heparinized blood was similar to that in citrated blood; thus, the present findings are more likely related to the anticoagulant effect of heparin. However, other molecules such as hirudin and synthetic thrombin inhibitors are presently being tested to determine whether heparin per se has any specific effects on platelet function in the perfusion system. Preliminary results (unpublished) indicate that hirudin does not significantly differ from heparin with respect to platelet deposition on subendothelium (mild injury).

These experimental results with surface- and flow-related factors may have important clinical implications. In spontaneous plaque rupture (unstable angina) when the vascular lumen is considerably reduced by stenosis, wall shear rates and stresses will be increased over values typical of the unobstructed vessel. The combination of the modified rheological conditions (high shear rate at the stenosis), the blood procoagulant factors, and the nature of the exposed material (collagen type I of atherosclerotic or injured vessel) might interact to induce rapid irreversible growth of a thrombus onto the damaged area and lead to occlusion of the vessel. Other components of the plaque matrix may also have an important contribution to thrombosis and should be investigated. Exposure of plaque matrix may not only stimulate platelets and the coagulation system but may also present a topologically rough surface with the consequent induction of local flow disturbances and recirculation zones that could further serve as niduses for thrombus attachment.

Stabilization of thrombus by vWF. In the present study, we have demonstrated a defect in the ability of platelets to deposit on collagen fibrils exposed at high shear rates to either heparinized or native blood deficient in vWF (Figures 3, 5, and 6; Table 4). A similar defect has been recently reported in these vWF-deficient animals when their blood was exposed over a broad range of shear conditions to deendothelialized blood vessels and in the ability of human platelets from patients with vWD to adhere to normal rabbit subendothelium, umbilical artery, and endothelial matrix. The defect in platelet deposition onto collagen observed in the present study at high shear rates in both native and heparinized blood is probably related to the inability of platelet thrombus to withstand shear removal forces in the absence of vWF, reflecting a defect in platelet-platelet cohesion. These findings provide further evidence for the proposed role of vWF in mediating platelet-platelet interaction by vWF binding to GP IIb-IIIa on stimulated platelets. Also, they support a role for vWF in platelet-collagen association (adhesion), as has been recently reported by Sakariassen et al. in an in vitro system with citrated blood. It has been suggested recently that released vWF from platelets may enhance substantially the local concentration of vWF compared with fibrinogen and that the released vWF from platelet granules preferentially binds to GP IIb-IIIa on stimulation with thrombin. In our studies in heparinized blood, the lack of thrombin suggests a further inhibition of platelet deposition in addition to that attributable to the lack of vWF as indicated by the enhanced defect under these conditions (Table 5). Recent results from Loscalzo et al. indicate a potential stabilization mechanism through platelet binding to polymerizing fibrin, which has been shown to be mediated by vWF and the GP Ib site on platelets.

Interestingly, the animals with heterozygous vWD, who show intermediate levels of vWF (Table 1), gave values of platelet deposition that were not statistically different from those obtained in normal animals. This finding correlates with the lack of clinical bleeding and normal bleeding time observed with these animals. It has been recently reported that pigs with vWD subjected to coronary vessel stenosis by an external ring did not develop vascular occlusion in contrast to normal animals. In these studies, permanent cessation of flow was caused primarily by platelet thrombi blocking the narrowed areas. Our present studies demonstrating the role of vWF in platelet-platelet interaction at high shear rates help explain these earlier findings conducted under less well-defined conditions.

General Comments

In this study, we have demonstrated that flow and specifically the local shear rate conditions regulate the attachment, growth, and stability of platelet masses on collagen. Indeed, rheology mediates significantly the biochemical mechanisms involved in platelet–vessel wall interaction. The high molecular weight glycoprotein vWF has an important physiological role on the stabilization of thrombus masses.
by securing platelet aggregate formation to the underlying substrate.

Because stenotic regions, or areas around partially occluding lesions, result in locally altered shear rate conditions, attention should be directed to the potential risk of platelet activation and sudden thrombotic occlusion, mainly in those situations in which the coagulation enzymes and zymogens are freely available. Also, the present findings suggest that immunologic blocking of this vWF protein may be a possible mechanism for the acute inhibition of thrombosis associated with the rupture of coronary atherosclerotic plaques or after balloon angioplasty. Studies are currently underway to test this hypothesis.

Acknowledgments

We thank A. Galvez, MD, J.A. Rosemark, BA, and E. Mogilevsky, VDD, for their collaboration and C. Busnadiego for the preparation of the manuscript. We also thank E.J. Bowie, MD, and D. Fass, PhD, for their continuous collaboration and support.

References

43. Behnke O: Platelet adhesion to native collagens involves proteoglycans and may be a two-step process. Thromb Haemost 1987;58:786–789
49. Badimon L, Badimon JJ, Turitto VT, Fuster V: Role of von Willebrand factor in mediating platelet-vessel wall interaction at low shear rate: The importance of perfusion conditions. Circulation 1988;78:

KEY WORDS • platelets • collagen • heparin • von Willebrand factor
L Badimon, J J Badimon, V T Turitto, S Vallabhajosula and V Fuster

Circulation. 1988;78:1431-1442
doi: 10.1161/01.CIR.78.6.1431

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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