von Willebrand's Disease Prevents Occlusive Thrombosis in Stenosed and Injured Porcine Coronary Arteries

TIMOTHY C. NICHOLS, DWIGHT A. BELLINGER, TIMOTHY A. JOHNSON, MARY ANN LAMB, AND THOMAS R. GRIGGS

SUMMARY We studied the role of von Willebrand factor in coronary thrombosis in normal, heterozygous, and homozygous von Willebrand's disease pigs by producing coronary stenosis with a Goldblatt clamp positioned around the left anterior descending coronary artery. Flow velocity was assessed by a 20-MHz Doppler velocity probe distal to the Goldblatt clamp. Myocardial extracellular potassium levels were measured by potassium-sensitive electrodes in myocardium supplied by the left anterior descending artery. Whereas stenosis sufficient to block reactive hyperemia to a 20-second occlusion produced an elevation of myocardial extracellular potassium, it produced neither spontaneous cyclic flow reductions nor permanent cessation of coronary blood flow velocity. Injury of the coronary artery at the stenosis site with spring-loaded forceps produced cyclic flow reductions or permanent cessation of flow in eight of nine phenotypically normal pigs. On the other hand, flow variations occurred in none of the 10 von Willebrand's disease pigs, including four given purified von Willebrand factor at a dose that failed to correct the bleeding time (p<0.001, t² test). Permanent cessation of flow was caused by an occlusive platelet-fibrin-red-blood-cell thrombus. Scanning electron micrographs from pigs with cyclic flow variations and from von Willebrand's disease pigs showed injured endothelium covered by adherent platelets, red and white blood cells, and fibrin. These data suggest an important role of native von Willebrand factor in sudden occlusive arterial thrombosis following stenosis and intimal injury. (Circulation Research 1986;59:15-26)

KEY WORDS • von Willebrand's disease • coronary thrombosis

VON WILLEBRAND factor (vWF) supports platelet adhesion to vascular subendothelium at high shear rates, >1600/sec, and is thereby important in hemostasis. A role for vWF in arterial thrombosis is not well established. Such a role would not be suspected initially since flow in large arteries creates low shear rates, <1000/sec. However, there is a growing body of evidence that conditions exist under which vWF contributes to arterial platelet activation and thrombosis. First, pigs with von Willebrand's disease (vWD) are known to be protected from the development of aortic atherosclerosis. Some studies suggest that this protection is related to reduced platelet adherence at the injured vascular wall and the resultant absence of release of platelet-derived growth factor. Second, there is evidence of retarded activation of the platelets in bleeder pigs, although the density of adherent platelets on injured coronary intima is similar in normal and vWD pigs. Third, myocardial infarctions may occur less frequently in vWD pigs with the same degree of coronary atherosclerosis as in normals.

Whether the role of vWF in arterial thrombosis is dependent on shear rate is not certain. However, clinical coronary thrombosis rarely occurs in the absence of preexisting stenosis from atherosclerosis. Stenosis might, in turn, affect the shear rate in an artery so that platelet activation and adhesion would be influenced by vWF, even in arterial segments that normally have low shear conditions.

An association of stenosis and occlusive thrombosis has been confirmed in canine and porcine models by Folts and others. The investigators have shown cyclic reductions in coronary blood flow (CFR) when plastic clamps are placed on the coronary arteries. If permanent cessation of flow (PCF) occurs, shaking or poking the stenotic area of the coronary artery can restore the CFR pattern. If the animal is killed at the nadir of flow during the CFR, the stenotic area of the vessel contains a platelet plug, as demonstrated by histological examination. Aspirin, ibuprofen, and indomethacin inhibit the CFR phenomenon, whereas heparin has no effect. Selective thromboxane synthetase inhibitors, prostacyclin, α-adrenergic blockers, serotonin blockers, nicergoline (an α-adrenergic blocker and platelet phospholipase inhibitor), and pos-
sibly, modulation of the autonomic nervous system, have all been shown to decrease or abolish CFR in artificially stenosed coronary arteries.\textsuperscript{11, 13-23} Although all of these agents affect platelet function, they alter endothelial receptors and metabolism as well.\textsuperscript{24} Further, in the canine model, studies of isolated coronary segments from the site of stenosis have shown an imbalance of thromboxane A\textsubscript{2} and prostacyclin, which could promote vasoconstriction and platelet aggregation.\textsuperscript{25} In none of these studies has the role of vWF in the cyclic flow reduction phenomenon been investigated.

We designed this experiment to determine whether vWF was critical to the development of CFR or PCF in pigs. Our results show a significantly reduced incidence of CFR and PCF in vWD pigs, and they support a hypothetical model of the process of arterial thrombosis that assigns an important role to the presence of von Willebrand factor.

**Materials and Methods**

**Animal Selection**

Nine phenotypically normal pigs and 10 pigs with von Willebrand’s disease were obtained from the closed colony of pigs at the Francis Owen Blood Research Laboratory. The disease in these pigs has been described previously.\textsuperscript{26} Animals with vWD had less than 1% of normal levels of vWF (Allain et al., Table 1). The phenotypically normal animals included five genotypic normals as well as four heterozygotes, which show no bleeding tendencies. The procedure for classifying these animals according to phenotype has been described.\textsuperscript{27} Both male and female animals were used. Ages ranged from 3 to 5 months, and weights were between 19 and 45 kg (Table 4). All animals were treated according to the standards set in the “Guide for the Care and Use of Laboratory Animals.”

**Anesthetic Administration**

All animals were initially sedated with ketamine-HCl (10 mg/kg), intramuscularly. Anesthesia was induced with a mixture of halothane (2-3%), 30% nitrous oxide, and 70% oxygen. The three agents were delivered in a system utilizing a 3-liter reservoir bag. When an animal was anesthetized sufficiently to suppress the eyelash response, it was intubated and placed on positive pressure ventilation. For the rest of the experiment, anesthesia was maintained with 1.5-2% halothane and oxygen. A seven-lead electrocardiogram then was obtained.

**Surgical Preparation and Instrumentation**

The femoral artery and vein were isolated by an inguinal incision and cannulated with 8F Teflon introducers. The venous line was used to infuse fluids and draw blood samples, and the arterial line was used to monitor arterial pressure and blood gases. The arterial line was connected to a P23GB Statham pressure transducer which was calibrated by a manometer. After induction of anesthesia and placement of catheters, the chest was opened via a median sternotomy, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was dissected free of surrounding tissue in two areas, each approximately 0.5-1.0 cm long. Side branches and crossing veins were ligated if needed. A 5-mm Goldblatt clamp was placed on the proximal LAD dissection site. Coronary artery flow velocity was monitored at the distal dissection site with a 20-MHz Doppler ultrasonic crystal applied to the vessel in such a way that flow was not impaired (Figure 1). The Doppler crystal was a 1-mm\textsuperscript{2} piezoelectric crystal embedded in epoxy in order to make a 45-degree angle with the direction of blood flow. This Doppler crystal was energized by a range-gated pulsed-Doppler unit. The unit was custom designed and manufactured by C.J. Hartley, Baylor University. It generated 20-MHz pulses with a repetition frequency of 62.5 kHz. The signal was range gated to maximum clarity.

Miniature K\textsuperscript{+}-sensitive plunge electrodes were placed in the myocardium supplied by the LAD distal to the Goldblatt clamp. These were used to measure myocardial extracellular K\textsuperscript{+} level ([K\textsuperscript{+}]c) as a marker of ischemia. The design and characterization of the electrodes have been described in a previous publication.\textsuperscript{28} Briefly, the electrodes were fashioned from Teflon-coated silver wire, 0.005 inch in diameter. The cut end of each wire was chloridized in sodium hypochlorite, covered with a mixture of cellulose acetate and titanium dioxide, soaked in 3.0 mM KCl solution, and covered with a polyvinyl-chloride-based membrane containing the K\textsuperscript{+} ionophore, valinomycin. Reference electrodes, similarly constructed, lacked only the ion-selective membrane. A K\textsuperscript{+}-sensitive and common refer-
trodes were amplified by a high impedance instrumentation amplifier serially connected to a low pass filter (1 kHz).

Aortic pressure, lead II of the ECG, [K\(^+\)], and Doppler flow velocity were recorded continuously on a model 7 Grass recorder. Sample values taken every 5 minutes were used for analysis. Arterial blood gases were obtained and ventilator adjustments made to maintain PO\(_2\) > 100 mm Hg, Pco\(_2\) between 35 and 45 mm Hg, and pH between 7.35 and 7.45.

**Coronary Stenosis**

After a 30-minute period of stabilization of the system, the Goldblatt clamp was closed to a degree sufficient to block reactive hyperemia. A 20-second total occlusion with a cotton-tipped swab applied directly to the LAD just proximal to the clamp was used to induce reactive hyperemia and to confirm its blockade.\(^{28, 29}\) With this accomplished, a 30-minute period of observation for cyclic flow reductions (CFR) or permanent cessation of flow (PCF) was undertaken. If either CFR or PCF was seen, the procedure was stopped and the heart was perfused (see “Histological and Ultrastructural Studies”). If neither CFR nor PCF occurred, the Goldblatt clamp was released just after a second 20-second occlusion confirmed that reactive hyperemia had been blocked throughout the period of observation.

**Coronary Artery Injury**

After blood flow velocity and [K\(^+\)] had stabilized with the Goldblatt clamp open, the LAD was injured in the area where the clamp was applied. The injury was induced by 2 or 3 occlusions of the artery with a spring-loaded forceps (Castroviejo Needle Holders). After 10 minutes of observation, the Goldblatt clamp again was closed enough to block reactive hyperemia. If CFR or PCF occurred before or after the clamp was closed, the experiment was stopped. If, within 30 minutes, neither CFR nor PCF was seen, the clamp was opened and, after stabilization, the injury was repeated. If flow velocity remained unchanged, the Goldblatt clamp was tightened a third time sufficient to block reactive hyperemia. After a final 30-minute period of observation, the procedure was terminated by giving the animal an overdose of sodium pentobarbital, and the heart was perfused as described below.

**von Willebrand Factor Preparation and Infusion**

von Willebrand factor was purified by described techniques to a final concentration of 2.2–6.9 μ/ml.\(^{30}\) A total of 546–1,518 U of purified vWF were diluted in approximately 500 ml of normal saline and given intravenously over 30 minutes to four animals (Nos. 16–19). Administration was completed by the beginning of the 30-minute period of stabilization prior to stenosis. The protocol was the same for the four transfused animals as for the others, except that blood samples were taken for plasma vWF levels and bleeding times\(^{31}\) determined prior to and at 30-minute intervals during the experiment.

**Histological and Ultrastructural Studies**

After each pig was killed, its heart was removed immediately and perfused with 0.15 M phosphate buffer followed by 1% gluteraldehyde and 4% formaldehyde at 100 mm Hg constant pressure. Pressure and flow were maintained in the coronary arteries by perfusing through a cannula in the left ventricle and clamping the pulmonary artery and veins, the caudal and cranial venae cavae, and the aorta. Drainage was via a small exit in the right atrium. After being perfused, the heart was immersed in fixative.

Histological sections were taken after at least 24 hours of fixation. The LAD was cut perpendicular to its long axis into segments of approximately 1 cm. Particular attention was paid to the sections of the LAD where the Goldblatt clamp and Doppler crystal had been located. The proximal portions of selected sections were processed for transmission electron microscopy. The middle portion of each section was opened longitudinally and prepared for scanning electron microscopy. The distal portion of each section was processed for light microscopy. Two sections were cut from each block and stained with either hemotoxalin and eosin or Verhoeff van Gieson stain.

Specimens were prepared for scanning electron microscopy by dehydration through a graded series of alcohols, critical point dried in liquid CO\(_2\), and coated with gold palladium. They were viewed with an ETEC scanning electron microscope (SEM).

Specimens for transmission electron microscopy were postfixed in osmium tetroxide, dehydrated in alcohol, and embedded in Epon. Sections were viewed with a Zeiss 109 transmission electron microscope.

**Morphometric Analysis of Stenosis and Injury**

Measurements of the internal and external elastic laminar and luminal perimeters were made on the Verhoeff-van-Gieson-stained sections projected onto the screen of a Ziess videoplan automatic image analyzer. From these measurements, the cross-sectional areas of the lumen, media, and total vessel were calculated. The percent stenosis at the Goldblatt clamp site was calculated using the mean of the luminal areas of the adjacent sections as the denominator in the following formula:

\[
\text{% stenosis} = 1 - \frac{\text{luminal area at clamp site}}{\text{mean of adjacent luminal areas}} \times 100\%
\]

The amount of injury to the artery at the clamp site was assessed by examination of both light and scan-
ning electron microscopic sections. The light micro-
scopic cross section was divided into eight equal
segments, and the number of segments that had pale-
staining smooth muscle cells, with or without pyknotic
nuclei, was recorded. The area of hemorrhage within
the media or between the media and adventitia was
measured with a Zieiss videoplan automatic analyzer.
This area then was expressed as percent of the total
medial area. We estimated the degree of disruption of
internal elastic lamina (IEL) by measuring the length
of vessel lumen where the IEL was absent, and ex-
pressing this length as the percent of the total length
of IEL and lumen boundary. The luminal surface area
displayed by each SEM section from the clamp sites
was measured from photomicrographs using the Zieiss
videoplan. The areas covered by (1) thrombus (aggre-
gations of platelets, red and white blood cells, and
fibrin), (2) exposed subendothelium (completely de-
nuded of endothelial cells with a single layer of at-
tached platelets), (3) partially denuded endothelium
containing islands of damaged but adherent endothe-

dial cells with a single layer of attached platelets, and (4)
intact endothelium were measured and expressed as a
percent of total surface.

Statistical Methods of Analysis
All measured variables were reported as means ±
SE. Comparisons between means for bleeders and nor-
mals were made using the Student's t test or χ² test.

Results

Flow Velocity After Stenosis and Injury
Eight of nine normal and heterozygous pigs had
CRF, PCF, or both, after the injury (Table 1, pigs
1–9). The CFR seen in these pigs were characterized
by a decrease in flow velocity over several minutes,
then an abrupt return to levels equal or nearly equal to
those prior to the beginning of the CFR (Figure 2). In

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<td>Mean ± SE</td>
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<td>85.5 ± 6.9</td>
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*Goldblatt clamp indicates that the clamp was either open (O) or stenosed (S) at the end of the experiment. In animal
No. 3, PCF was seen after the first injury. The clamp was opened and CFR appeared.
†The difference in flow velocity changes following stenosis and injury is statistically significant (8/9 normals and heterozygous vWD vs. 0/10 vWD with and without purified vWF, p < 0.001, χ² test). vWF = von Willebrand factor; vWD = von Willebrand's disease.
‡For the vWD animals given vWF, the first value is the preinfusion and the second is the average vWF level obtained
on multiple plasma samples taken throughout the stenosis and injury period.
those pigs with PCF, the flow abruptly stopped and did not return (Figure 3), except in animal No. 3. In this animal, PCF occurred after the first injury with stenosis present; when the stenosis was released, flow reappeared with ensuing CFR, and the experiment was terminated. One normal (No. 2) and one heterozygous pig (No. 6) had CFR after the first injury while the clamp was open; the heterozygote (No. 6) then proceeded to have PCF. A second heterozygote (No. 8) had CFR after the second injury. Two normal pigs (Nos. 1 and 4) had PCF after the first injury; another normal and one heterozygote (Nos. 5 and 9) exhibited PCF after the second injury. The Goldblatt clamp was open in these four cases. Only one pig (No. 5) required two injuries and repeat stenoses before PCF occurred. The one pig without flow velocity changes, a heterozygote (No. 7), had histological findings similar to those with CFR (vide infra).

None of the 10 pigs with von Willebrand’s disease exhibited CFR or PCF following stenosis with or without injury. This included four pigs given purified vWF. This difference in the incidence of flow velocity changes between the two groups of pigs following injury was statistically significant (8/9 normal and heterozygous vWD vs. 0/10 homozygous vWD, \( p < 0.001 \), \( \chi^2 \) test).

corrections.

### von Willebrand Factor Levels After Infusion and Bleeding Time Results

The average plasma vWF level of infused pigs ranged from <1% to 89% (Table 1); however, the prolonged bleeding times were not corrected in three of the four pigs receiving infusions of purified vWF. In animal No. 16, the bleeding time shortened from >10 minutes to 7.5 minutes with a peak vWF level of 79%. This bleeding time was still twice the values for normal and heterozygous pigs of <3 minutes.

### Histology and Ultrastructure

Normal pigs exhibiting PCF had occlusive thrombi (Figure 4), and those with CFR had nonocclusive thrombi (Figure 5). These thrombi were composed of platelets, red and white blood cells, and fibrin. In normal and heterozygous pigs with CFR and in vWD
FIGURE 4. Scanning electron micrographs of any occluding thrombus (T) at the injury site in the LAD from a heterozygous pig (No. 6) with PCF. Panel A: section of LAD cut longitudinally. Dashes indicate leading edge of a large thrombus that fills the vascular lumen (original magnification 80 ×). Sketch of vessel with clot included on insert. Panel B: scanning electron photomicrograph of thrombus at higher magnification (original magnification 1200 ×). Fibrin and RBC predominate in this portion of the thrombus.
FIGURE 5. Scanning electron photomicrograph of LAD at the injury site of a heterozygous pig (No. 8) with cyclic flow reductions (CRF). Panel A: the surface is covered with numerous platelets and white blood cells. A portion of disrupted tissue is at the upper right (original magnification 400×). Panel B: higher magnification of platelets and white blood cells forming a dense covering over the exposed subendothelium.
pigs, the exposed subendothelium was covered with a carpet of platelets, red blood cells, and fibrin (Figures 5 and 6). Light microscopy of sections stained with hemotoxylin and eosin showed evidence of hemorrhage into the tunica media of the injured segment in both phenotypes (Figure 7).

**Coronary Stenosis and Injury**

The coronary artery segments at the clamp in the vWD pigs (Nos. 10–19) had an average $81.5 \pm 4.5$ stenosis induced by the clamp during inhibition of reactive hyperemia (Table 1). The mean percent stenosis at the injury site for animals terminated with the clamp open was $44.5 \pm 12.6$ (Pigs 1, 2, 3, 4, 8, and 9). For Pig 6, the section of LAD for calculation of percent stenosis was lost in processing.

The coronary artery segments at the clamp sites are given in Tables 2 and 3. There were no differences between the two groups in number of sections with smooth muscle cell damage ($p = 0.66$), percent medial-adventitial hemorrhage ($p = 0.65$), and percent disrupted IEL ($p = 0.61$). In normal pigs, a greater percent of the surface was covered by thrombus than in vWD pigs ($p < 0.05$). However, the vWD animals had significantly more subendothelium not covered by thrombus ($p < 0.01$). Based on light and SEM sections, the thrombus overlaid all subendothelium completely denuded of endothelial cells. When the areas covered by thrombus and exposed subendothelium were combined, there was no difference between bleeders and normals ($p = 0.9$). When combined, these areas represent the total extent of injury to the vessel.

**Myocardial Extracellular Potassium Levels**

The myocardial extracellular potassium level ($[K^+]_c$) increased approximately 10% above baseline when flow velocity was decreased to sufficient levels to inhibit reactive hyperemia. The $[K^+]_c$ also increased further in the phenotypic normal animals during permanent cessation of flow (Figure 3). However, the $[K^+]_c$ was not consistently altered during cyclic flow reductions. In fact, wide variations of flow velocity occurred during CFR without causing an increase in $[K^+]_c$ (Figure 2).

**Hemodynamic and Hematological Comparisons**

When normal and vWD pigs were compared, no statistically significant differences in systolic, diastolic, or mean blood pressures, or in heart rate were detected (Table 4). vWF was absent from pigs with vWD and present in normals and heterozygotes with an average level of $83.8\% \pm 10.8$ in Pigs 1–9.

**Animal and Heart Weights and Coronary Artery Size**

Animal and heart weights were not statistically different. Further, the mean planimetric coronary artery

**FIGURE 6. Scanning electron photomicrograph of nonocclusive thrombus present at the injury site in an animal with von Willebrand's disease (vWD pig No. 13, original magnification 700×). The thrombus is composed of platelets, red and white blood cells, and fibrin.**
lumen sizes just proximal and distal to the clamp were equivalent (Table 4).

Discussion

Sudden reductions in coronary blood flow velocity were seen exclusively in phenotypically normal pigs (genotypic wild type and heterozygotes), and only after pinch-injury to the coronary artery. In marked distinction, vWD pigs treated in the same manner never demonstrated these coronary blood flow velocity changes \( (p < 0.001, \chi^2 \text{ test}) \). The reductions in coronary blood flow velocity seen in normal pigs occurred in two forms, cyclic flow reductions and permanent cessation of flow. PCF was caused by persistently occlusive thrombi. CFR have been shown to be caused by transient luminal occlusion by platelet aggregates which are cyclically dislodged and embolized downstream. Our experiment was terminated when CFR or PCF occurred as a stable pattern, and, thus, the vessels from pigs with CFR showed only the remains of occlusive platelet aggregates. Whereas vWD pigs had platelets adhering to injured endothelium that morphologically resembled that seen in normal pigs with CFR, there were no flow velocity changes or \([K^{-}]\), changes to indicate vWD pigs generated occlusive thrombi during the experiment. These findings are in agreement with previous studies that have shown a role of platelets in the production of CFR/PCF. However, our study is the first to show an effect of vWF in the creation of CFR/PCF.

The exact role of vWF in CFR/PCF is complex and could not be fully determined in this study, since transfusion of purified vWF did not correct bleeding times or produce CFR/PCF. Failure of purified vWF to correct the bleeding time has been previously reported and is consistent with our experience and that of others. This phenomenon is probably related to changes in the molecular conformation of the functional components of vWF occurring during purification. It is also possible that vWF from platelets, endothelium, or subendothelium, rather than, or in addition to, circulating vWF, is required for correction of bleeding times and/or the production of CFR/PCF. Most information about vWF function is derived from in vitro models that mimic microcirculation. This approach has shown that the significance of the defect in vWD is directly affected by the conditions of local blood flow. These models have seen effects of vWF only at high shear rates, \( >1600/\text{sec.} \). These shear rates are much higher than the 300–1000/sec range that would be expected in normal coronary arteries. These in vitro findings make it improbable that vWF would affect platelet adhesion to the injured endothelium of the epicardial coronary arterial circulation. In this study, a platelet carpet was seen at sites of endothelial injury in all animals without occlusive thrombi. This observation is consistent with normal platelet adhesion to coronary subendothelium in the bleeder pigs. However, platelet macroaggregation and thrombosis, rather than adhesion, was the focus of our study.
TABLE 2. Morphological Evaluation of Vessel at Injury Site—Light Microscopy

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</tr>
<tr>
<td>Mean ± SE</td>
<td>7.8 ± 0.17</td>
<td>13.5 ± 4.9</td>
<td>35 ± 15.2</td>
</tr>
<tr>
<td>Homozygous vWD Given Purified vWF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>17</td>
<td>8</td>
<td>17</td>
<td>38</td>
</tr>
<tr>
<td>18</td>
<td>7</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>19</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>4.5 ± 1.8</td>
<td>4.3 ± 4.3</td>
<td>19 ± 7.2</td>
</tr>
</tbody>
</table>

There are no significant differences between the two groups (Student's t test).

*Expressed as a percent of total medial area.
†Expressed as a percent of total IEL length.

Several lines of evidence suggest that vWF has a role in supporting platelet-to-platelet aggregation. The first such suggestion came from the observation that the antibiotic ristocetin would support platelet aggregation in plasma only in the presence of vWF.40 In the porcine system, normal pig plasma will cause aggregation of human platelets with or without the addition of ristocetin, whereas porcine vWD plasma will not.20 Recently, Turitto and Weiss have shown defective formation or a reduction in the size of platelet thrombi on everted artery segments in the annular perfusion chamber when the chamber was perfused with blood from patients with von Willebrand's disease. Whereas platelet adhesion abnormalities again were seen at high shear rates, quantitatively smaller platelet thrombi were seen at lower shear rates, <1300/sec, compared to normal controls and patients with classic hemophilia A. This reduced platelet thrombus size occurred at a shear rate at which platelet-to-subendothelial attachment in vWD is normal.10 This defec-

tive platelet thrombus formation may be due to abnormalities of platelet-to-platelet interactions important for thrombus formation and growth. It is also possible that the absence of vWF alters platelet activation in such a way that the release of proaggregatory substances such as thromboxane and serotonin does not occur normally.8,23

In a previous in vivo study from our laboratory, a possible role of vWF in porcine coronary thrombosis was found. We showed no myocardial infarctions among 14 homozygous vWD pigs with coronary atherosclerosis of a degree similar to that in a matched group of normals and carriers. There were five infarcts among 24 pigs in the normal and carrier group.9 There were no thromboses in the pigs that did have infarctions, but any thrombi would have had time to lyse.

Our findings document the absence of cyclic and
permanent flow velocity reductions in stenotic and injured coronary arteries of swine with von Willebrand’s disease. Further, in vWD animals, significantly less of the damaged endothelium was covered by thrombus. These observations are consistent with the hypothesis that von Willebrand factor supports platelet-to-platelet aggregation and thrombosis in moderate-sized arteries and may play a role in clinical coronary thrombosis.

Two additional observations in this study deserve comment. First, CFR/PCF in our experiments could not be induced by stenosis alone. However, injury induced after 30 minutes of stenosis was followed by flow velocity aberrations. Thus, occlusive thrombosis following vessel injury seemed to occur exclusively in the setting of recently ischemic myocardium and vessel wall. It is possible that the influence of vWF on thrombosis was enhanced in the setting of ischemia. A second important observation was that the intramyocardial potassium activity was frequently unchanged during well-documented alterations in coronary flow velocity. This implies that cyclic thrombotic events occur without evidence of myocardial injury.

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References

1. Tschopp T, Weiss HJ, Baumgartner HR: Decreased adhesion of platelets to subendothelium in von Willebrand’s disease. J Lab Clin Med 1974;83:296-300
geline, a platelet-active alpha-adrenergic antagonist. J Am Col
Cardiologists 1984;3:1417-1426
cyclin contributes to the efficacy of a thromboxane synthetase
inhibitor for preventing coronary artery thrombosis. J Pharma-
col Exp Ther 1981;219:299-308
din and thromboxane production in a canine model of myocard-
24. Hogan AG, Muhrer ME, Bogart R: A hemophilia-like disease
paraformaldehyde: A new reagent for assay of von Willebrand
factor and platelet aggregating factor. J Lab Clin Med 1975;
85:318-326
factor: Gene dosage relationships and transfusion response in
bleeder swine — a new bioassay. Proc Natl Acad Sci USA
1974;71:2087-2090
electrodes for online determination of intravascular and myo-
28. Fedor JM, McIntosh DM, Rembert JC, et al: Coronary and
regional myocardial blood flow response to transient ischemia
velocity and reactive hyperemia in the coronary circulation of
30. Olson JD, Brockway WJ, Fass DN, et al: Purification of por-
cine and human ristocetin-Willebrand factor. J Lab Clin Med
1977;89:1278-1294
31. Mertz ET: The anomaly of a normal Duke’s and a very pro-
longed bleeding time in swine suffering from an inherited
factor concentrate therapy in von Willebrand disease: Dissocia-
tion of bleeding-time factor and ristocetin cofactor activities.
JAMA 1976;236:2770-2772
33. Chediak JR, Telfer MC, Green D: Platelet function and im-
munologic parameters in von Willebrand’s disease following
cryoprecipitate and factor VIII concentrate infusion. Am J Med
1977;62:369-376
factor VIII complex: Functional and structural heterogeneity
observed in von Willebrand swine with transfusion. Proc Natl
Acad Sci USA 1977;74:759-763
35. Bowie EJW, Fass DN, Owen CA Jr. Hemostatic effect of
transfused Willebrand factor in porcine von Willebrand’s dis-
factor in the vessel wall mediates platelet adherence. Blood
1985;65:85-90
Willebrand factor in subendothelium mediates platelet adhe-
sion. Blood 1985;65:823-831
38. Baumgartner HR, Mugglie R, Tschopp TB, et al: Platelet ad-
hesion, release and aggregation in flowing blood: Effects of
surface properties and platelet function. Thrombos Haemostas
1976;35:124-138
39. Turitto VT: Blood viscosity, mass transport and thrombogene-
sis. Prog Hemostasis Thromb 1982;6:139-178
40. Howard MA, Firkin BG: Ristocetin — a new tool in the inves-
tigation of platelet aggregation. Thromb Diath Haemorrh
1971;26:362-369
von Willebrand's disease prevents occlusive thrombosis in stenosed and injured porcine coronary arteries.
T C Nichols, D A Bellinger, T A Johnson, M A Lamb and T R Griggs

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