The Roles of von Willebrand Factor and Factor VIII in Arterial Thrombosis: Studies in Canine von Willebrand Disease and Hemophilia A

By Timothy C. Nichols, Dwight A. Bellinger, Robert L. Reddick, Scott V. Smith, Gary G. Koch, Keir Davis, Jeff Sigman, Kenneth M. Brinkhous, Thomas R. Griggs, and Marjorie S. Read

We have studied the roles of von Willebrand factor (vWF) and factor VIII in arterial thrombosis in four canine phenotypes: normal (n = 6), hemophilia A (n = 11), von Willebrand disease (vWD) (n = 9), and hemophilia A/vWD (n = 1). vWF activity was determined by botrocetin-induced agglutination of fixed human platelets and vWF antigen (vWF:Ag) by Laurell electroimmunoassay and crossed immunoelectrophoresis. Plasma from normal dogs and those with hemophilia A had vWF activity, vWF:Ag, and a full range of vWF:Ag multimers on gel electrophoresis equivalent to normal canine plasma pool. Platelet cytosol contents were isolated by freezing and thawing, triton X-100 solubilization, or sonication of washed platelets with and without protease inhibitors and inhibitors of platelet activation. Washed platelets were also stimulated with calcium ionophore and MgCl₂. There was no measurable vWF activity or vWF:Ag in platelet lysates or releasates in any dog regardless of phenotype. All dogs were studied using a standard arterial stenosis and injury procedure to induce arterial thrombosis. Thromboses were detected by cyclic reductions in Doppler blood flow velocity. Vessels were examined by light and scanning electron microscopy. Thrombosis developed in the arteries of normal (9 of 10) and hemophilia A dogs (16 of 16) but in none of the vWD dogs (0 of 10). Infusion of canine vWF cryoprecipitate into vWD dogs markedly shortened bleeding time but did not support thrombosis as seen in dogs with vWF in the plasma and subendothelium. Thrombosis, then, fails to occur when vWF is absent from the plasma and subendothelial compartments or present only in the plasma compartment. These data are consistent with the hypothesis that vWF in the plasma and subendothelium supports thrombosis. Neither plasma FVIII nor platelet vWF is essential for thrombosis in this model.

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VON WILLEBRAND FACTOR (vWF) is a large adhesive glycoprotein present in endothelial cells and subendothelium in humans, pigs, and dogs. vWF is also present in human platelets and porcine platelets. There are conflicting reports about whether or not canine platelets contain vWF. A decrease in the degree of expression of plasma or platelet vWF in humans can produce a bleeding diathesis. In addition, high molecular weight vWF multimers present in human endothelial cells and platelets may be responsible for part of the biologic activity of vWF ex vivo. Variations in both the compartmentalization and multimeric distribution of vWF within these compartments, then, can alter the phenotype of a given species and may be important for the function(s) of the vWF protein.

vWF has several complex roles in hemostasis and thrombosis. At sites of exposed subendothelium, vWF supports the initial adhesion of platelets functioning as a ligand between platelet membrane glycoproteins and the subendothelium. This adhesive property of vWF is particularly evident at high shear rates. Once platelets attach to the subendothelium, vWF supports platelet spreading in pigs. If the vessel is injured at a stenotic site, vWF is required for the development of occlusive thrombosis. The relative contribution of vWF in plasma, platelets, endothelium, and subendothelial matrix in platelet attachment and spreading and arterial thrombosis is only partly understood and may differ between species. vWF serves as a carrier for factor VIII (FVIII) in plasma in humans, pigs, and dogs. This carrier function may provide for the delivery of FVIII to sites of arterial injury. Such a series of events could both localize FVIII activity where vWF attaches to exposed subendothelium and promote thrombosis. However, a monoclonal antibody raised to purified porcine vWF, BB3-BD5, both prevents and aborts thrombosis by neutralizing vWF when infused into normal pigs but does not affect FVIII activity. These data suggest that vWF supports thrombosis independent of its function as a carrier for FVIII. Studies have not been done in vivo in the absence of FVIII to confirm this finding.

The purpose of this study was to examine the relative roles of plasma and platelet vWF and FVIII in induction of arterial thrombosis in dogs. These studies were performed on the Chapel Hill strains of dogs with normal, hemophilia A, von Willebrand disease phenotypes, and a newly developed strain of bleeder dogs with combined defects, hemophilia A/vWD. No platelet vWF was detected in these dogs regardless of phenotype. Thrombosis developed in 90% of stenosed and injured arteries in normal dogs but in none of the vWD dogs even when they were infused with vWF concentrates. Replacement of plasma vWF by infusion, then, does not support thrombosis in vWD dogs as seen in dogs that have vWF within the plasma and subendothelium. Thus, thrombosis fails to occur when vWF is absent from the plasma and subendothelial matrix or present only in the plasma compartment. Moreover, thrombosis occurred in hemophilia A dogs as readily as in normal dogs. vWF, then, supports thrombosis independent of its association with FVIII and when it is absent.
from platelets. These data are consistent with the hypothesis that vWF in the plasma and subendothelium supports thrombosis. These data have been reported in part.33

MATERIALS AND METHODS

Experimental animals. Normal (n = 2), hemophilia A (n = 11), vWD (n = 6), and hemophilia A/vWD (n = 1) dogs came from the closed colony at the Francis Owen Blood Research Laboratory at the University of North Carolina, Chapel Hill. Four additional normal dogs were purchased from the Division of Laboratory Animal Medicine, University of North Carolina. All animals were treated according to the standards set in Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23). No animal had received blood products within 2 weeks of these studies.

Dogs with hemophilia A had less than 1% FVIII activity but normal vWF antigen and activity levels and normal saline bleeding times.34 vWD dogs had less than 1% vWF activity and vWF:Ag, depressed levels of FVIII activity, and bleeding times longer than 15 minutes. The dog with combined hemophilia A/vWD had no detectable FVIII activity, vWF activity nor vWF:Ag, and bleeding time of greater than 15 minutes. Dogs were classified as phenotypically normal if they had (1) FVIII activity levels and plasma vWF activity and vWF antigen (vWF:Ag) levels ≥60% of that found in normal canine pooled plasma, and (2) normal bleeding times.

FVIII assays. FVIII coagulant activity was performed by a modified one-stage method using a kaolin-activated FVIII-deficient canine plasma substrate.35

Plasma and platelet vWF assays. Platelet-poor plasma (PPP) was prepared by centrifugation of blood anticoagulated with 3.2% sodium citrate, pH 6.8 (1/9 vol/vol, 2,000g, 5 minutes). PPP was either tested immediately or frozen at −70°C until assayed. Platelet-rich plasma (PRP) was prepared by centrifugation of blood anticoagulated with acid-citrate-dextrose (ACD): 100 mmol/L sodium citrate, 0.07 mol/L citric acid, 0.1 mol/L dextrose, pH 4.5, 7.5–4.25: vol/vol, 500g, 8 minutes). The platelets were washed three times by resuspension in citrated saline (0.9% NaCl, 5 mmol/L sodium citrate, pH 6.5) and repeat centrifugation. In some experiments, PRP was prepared from blood anticoagulated with EDTA (20 mmol/L) containing leupeptin (10 μmol/L) and prostaglandin E-1 (PGE-1) (50 ng/mL). In these experiments, the platelets were washed in citrated saline containing EDTA, leupeptin, and PGE-1 at the same concentrations as above.

Platelet lysates were prepared by three different methods. First, washed platelets were frozen and thawed three times. Second, washed platelets were solubilized by the addition of 20% Triton X-100. Third, washed platelets were disrupted by sonication (Branson Sonifier 250, Danbury, CT). With all three techniques, the platelet debris was separated from the lysate by centrifugation (2,000g, 10 minutes, 23°C). Some lysates were prepared in the presence of protease inhibitors and inhibitors of platelet activation (ACD with aprotonin 400 KIU/mL and 300 mmol/L PGE-1 or EDTA, leupeptin, and PGE-1 at the concentrations stated above). Normal pig and human platelets were used as positive controls for these methods.

In some experiments, washed canine platelets were stimulated to undergo release by the addition of calcium ionophore (A23187, 1 μmol/L and 100 nmol/L) and MgCl2 (0.02 mol/L). The stimulated platelets were removed by centrifugation (2,000g, 10 minutes, 23°C) and the supernatant was assayed for vWF activity and antigen.

Aliquots of the washed platelets were fixed in glutaraldehyde before and just after producing platelet lysates or releasates for examination by transmission electron microscopy (TEM) to confirm platelet disruption or degranulation.37 Plasma, platelet lysates, and platelet releasates were assayed for vWF activity using the macroscopic tap tube procedure with botrocetin and paraformaldehyde-fixed, iophylogel human platelets.38,39

Fixed human platelets are the standard platelet reagent for vWF assay in our laboratory. We have obtained comparable results with fixed human or fixed canine platelets in this assay.37 Appropriate dilutions were done to exclude an inhibitor of vWF activity. vWF:Ag was assayed by Laurell electroimmunoassay.40 The vWF:Ag multimer analysis was performed by sodium dodecyl sulfate (SDS) gel electrophoresis as described.41 The dry gels were scanned by laser densitometry for the distribution of vWF multimers with the LKB Ultrosan XL (Pharmacia LKB, Piscataway, NJ). Crossed immunoelectrophoresis (CIE) of plasma and platelet lysates was performed on 1% agarose gels with and without denaturing agents.42

rabbit anti-canine vWF antibody. A rabbit anti-canine polyclonal antibody was raised against canine vWF by the method of Lamb et al.43 Canine cryoprecipitate from the plasma of hemophilia A dogs was chromatographed on 4% agarose (Biogel A-15m, 200–400 mesh; Bio-Rad Laboratories, Richmond, CA). The void volume fractions were pooled and used as antigen for immunizations. The antigen was made monospecific for vWF by adsorption of 1 vol of antiserum with cryoprecipitate from 2 vol of plasma from a dog homozygous for vWD. This monospecific antibody did not inhibit canine FVIII activity (data not shown) and was used to detect vWF:Ag in all assays and to stain gels.

Canine plasma and platelet fibronectin. Canine fibronectin was measured in platelet lysates, platelet releasates, and PPP by enzyme-linked immunosorbent assay (ELISA) with a polyclonal rabbit anti-canine antibody.44 Fibronectin in the platelet lysates and releasates was used as a control for recovery of platelet α-granule contents.

Bleeding time. Saline ear bleeding times were performed on all dogs.45

Arterial stenosis and injury procedure: Animal preparation, histologic studies, and vessel morphometry. The stenosis and injury procedure was performed under general anesthesia and with physiologic monitoring as described.46–50 This model was modified from that reported by Folts and Uchida.45,46 Briefly, general anesthesia was maintained with halothane (1% to 3%) from a vaporizer (Flumotec Vaporizer, Foregger, Smithtown, NY). The vaporizer was periodically checked for calibration by a Type 18 Riken Gas Analyzer (A.M. Bickford, Wale City, NY).

Stenosis and injury were then applied to one or both of the carotid arteries and/or a coronary artery (left circumflex or left anterior descending) with a Goldblatt clamp. Thrombosis was detected by cyclic flow reductions in arterial blood flow velocity (Doppler, 20 MHz) following stenosis and injury. After each dog was killed with an overdose of pentobarbital, the heart was pressure perfused and placed in fixatives with the carotid arteries. The degree of arterial stenosis and injury was determined by computer-assisted planimetry of light microscopy arterial cross sections (Zeiss Videoplan). Coronary and carotid arterial injury was assessed by the amount of smooth muscle cell damage and disruption of the internal elastic lamina.46–50 The presence or absence of thrombosis was also determined from examination of arterial sections with light microscopy.

Isolation and infusion of vWF. Cryoprecipitate containing vWF but lacking FVIII (FVIII-deficient cryoprecipitate) was prepared from PPP of hemophilia A dogs.47 The cryoprecipitate was stored at −70°C and dissolved in a minimal volume of 0.9% NaCl just before infusion. Fresh normal canine PPP was prepared for infusion into one vWD dog: vWF activity, antigen concentration, and multimeric distribution were determined for each infusate. One unit of vWF activity is defined as that equal to the amount present in 1 mL of normal canine pooled plasma.

The products were infused over approximately 60 minutes after the surgical preparation had been completed and all baseline samples had been obtained but before the stenosis and injury procedure. Following vWF product infusion, the stenosis and injury procedure was
Table 1. Effect of Canine Phenotype, FVIII, and Platelet and Plasma vWF Activity and Antigen on the Production of Arterial Thrombosis

<table>
<thead>
<tr>
<th>Phenotype (n)</th>
<th>Infusate</th>
<th>Bleeding Time (min)</th>
<th>FVIII (%)</th>
<th>Platelet Count (mm$^3$ x 10$^9$)</th>
<th>Platelet$^*$</th>
<th>Plasma</th>
<th>Thrombosis per No. of Arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (6)</td>
<td>None</td>
<td>2.3 (1.5, 2.6)</td>
<td>94 (86, 122)</td>
<td>297.5 (135, 365)</td>
<td>&lt;1&lt;1</td>
<td>99 (61, 158)</td>
<td>85 (98, 124)</td>
</tr>
<tr>
<td>vWD (6)</td>
<td>None</td>
<td>&gt;15 (7.8)</td>
<td>15.5 (225, 275)</td>
<td>&lt;1&lt;1&lt;1&lt;1</td>
<td>&lt;1&lt;1&lt;1&lt;1</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>None</td>
<td>2.7 (1.3, 4.4)</td>
<td>&lt;1 (230, 260)</td>
<td>270 (295, 300)</td>
<td>&lt;1&lt;1</td>
<td>135 (87, 160)</td>
<td>110 (76, 152)</td>
</tr>
<tr>
<td>vWD (1)</td>
<td>FVIII-deficient canine cryoprecipitate</td>
<td>4.1 (1.3, 4.4)</td>
<td>17 (230, 260)</td>
<td>298 (295, 300)</td>
<td>&lt;1&lt;1</td>
<td>103 (44, 162)</td>
<td>41.5 (36, 44)</td>
</tr>
<tr>
<td>vWD (1)</td>
<td>Normal canine plasma</td>
<td>12 (1.3, 4.4)</td>
<td>22 (230, 260)</td>
<td>305 (295, 300)</td>
<td>&lt;1&lt;1</td>
<td>54 (1.3, 4.4)</td>
<td>67 (1.3, 4.4)</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>FVIII-deficient canine cryoprecipitate</td>
<td>&gt;15 (1.3, 4.4)</td>
<td>&lt;1 (230, 260)</td>
<td>355 (295, 300)</td>
<td>&lt;1&lt;1</td>
<td>15 (7, 80)</td>
<td>44 (225, 275)</td>
</tr>
<tr>
<td>A/vWD (1)</td>
<td>Normal canine plasma</td>
<td>&gt;15 (1.3, 4.4)</td>
<td>&lt;1 (230, 260)</td>
<td>355 (295, 300)</td>
<td>&lt;1&lt;1</td>
<td>15 (7, 80)</td>
<td>44 (225, 275)</td>
</tr>
</tbody>
</table>

vWF activity is expressed as a percentage of normal canine plasma pool. In dogs that received a vWF-containing product, the plasma vWF values given were those obtained after infusion and during the stenosis and injury procedure. Median values are listed with maximum and minimum values listed below in parentheses.

* Platelet vWF was determined in a subset of all groups: normal (n = 2), vWD (n = 5), hemophilia A (n = 6), hemophilia A/vWD (n = 1).

† The ratio indicates the number of arteries that developed thrombosis per total number of arteries tested per group. For example, 10 of 11 arteries thrombosed in the six normal dogs.

$^{*}$ P < .01, Wilcoxon rank sum.

begun. Bleeding time was repeated at 30-minute intervals during the stenosis and injury experimental procedure (2.5 to 3.0 hours). In addition, blood samples were also collected every 30 minutes for measurement of circulating vWF antigen, vWF activity, and the multimeric distribution of vWF:Ag at the time of the stenosis and injury procedure. Results of the bleeding time and vWF measurements reported for these animals are those obtained following product infusion and during the stenosis and injury protocol.

Statistical methods of analysis. All measured variables are reported as median, minimum, and maximum. The Wilcoxon rank sum test was used to compare the normal, vWD, and hemophilia A dogs in a pairwise manner for variables with ordinal or interval levels of measurement, and its Kruskal-Wallis extension was used for overall comparisons. Unless otherwise noted, P values are from these tests. Fisher’s exact test was used for comparisons concerning categorical variables. The association of measured variables with one another was evaluated with linear regression methods and Spearman rank correlation coefficients.

RESULTS

Canine plasma vWF, FVIII, and fibronectin. The results of measurements of plasma vWF and FVIII are shown on Table 1 for all dogs. Plasma from vWD dogs had less than 1% vWF activity and antigen. FVIII activity was reduced in the vWD dogs. Plasma from the hemophilia A dogs had less than 1% FVIII activity but normal levels of vWF activity, and antigen. Plasma from the hemophilia A/vWD dog had less than 1% vWF:Ag, vWF activity, and FVIII activity. The plasma of all normal dogs contained vWF activity, vWF:Ag, and FVIII equivalent to that found in normal canine pooled plasma. In addition, the distribution of vWF:Ag multimers was comparable to that in normal pool plasma (Fig 1). Plasma fibronectin was measured in dogs of the following phenotypes: normal (258 µg/mL, n = 1), hemophilia A (420 µg/mL, n = 1), and vWD dog (420 µg/mL, n = 1). These plasma fibronectin values are within the range reported for dogs.43

Canine platelet vWF and fibronectin. Platelet lysates and releasates were prepared by different physical and chemical procedures chosen to minimize the chance of destroying any extant platelet vWF during processing.45-50 Platelet lysates were prepared from blood anticoagulated with citrate or EDTA and in the presence of protease inhibitors (aprotinin

![Fig 1. Multimeric distribution of vWF:Ag in the platelets and plasma of normal (lanes 1 and 2), hemophilia A (lanes 3 and 4), and vWD (lanes 5 and 6) dogs. Platelet lysates are shown in lanes 1, 3, and 5. Plasma samples are shown in lanes 2, 4, and 6. Normal canine pool plasma is shown in lane 7 for comparison.](image-url)
or leupeptin) and a platelet membrane stabilizer (PGE-1). None of these procedures yielded detectable canine platelet vWF antigen or activity (Table 1), nor were any vWF multimers detected (Fig 1). Dilution studies failed to detect an inhibitor of vWF activity in canine platelets. In addition, no precipitin arc was seen on CIE of the platelet lysates of any canine phenotype (data not shown). This finding was in contrast to the precipitin arc detected on CIE of plasmas from normal dogs and hemophilia A dogs that was comparable to that found in normal canine pooled plasma (data not shown).

Platelet lysates prepared by these methods from normal pig and human platelets contained normal amounts of vWF activity and antigen and a normal distribution of multimers (data not shown). Complete disruption of platelets by each method (ie, freezing and thawing, Triton X-100, and sonication) was confirmed by transmission electron micrograph (TEM). Platelet degranulation following stimulation with MgCl2 and two concentrations of A23187 was confirmed by TEM taken before and after stimulation (data not shown). Finally, there was no detectable vWF functionally or antigenically in any of the platelet washes before stimulation or disruption.

The platelet lysates and releasates from all procedures yielded detectable fibronectin with an average of 5.2 ± 1.3 µg/mL X 10^10 platelets. These data suggest that platelet α-granule contents were successfully released and recovered by these physical and chemical methods.

Incidence of thrombosis by phenotype. The incidence of thrombosis in the four canine phenotypes is listed on Table 1. Ten of 11 arteries (6 coronary, 5 carotid) tested in 6 normal dogs developed cyclic flow reductions (CFR) indicating thrombosis was induced by stenosis and injury. In addition, CFR occurred in all 16 arteries (5 coronary, 11 carotid) tested in 11 hemophilia A dogs (Fig 2). In contrast, no CFRs were detected in any of 10 arteries (2 coronary, 8 carotid) in six vWD dogs (Fig 2). We have previously found that the incidence of thrombosis in these two arteries relative to the presence or absence of vWF is equivalent, and all dogs in this present study with assessment for both carotid and coronary arteries had the same outcome. This finding has allowed the grouping of the two circulations for statistical analysis. The difference between the incidence of thrombosis between phenotypes in the present study is significant (P < .01 for percentage of tested arteries with thrombosis per dog, Wilcoxon rank sum); this difference is also significant for carotids alone for which sufficiently many animals were assessed to allow separate testing.

Plasma vWF, bleeding time, and incidence of thrombosis after infusion of vWF. FVIII-deficient cryoprecipitate was infused into two vWD dogs (336 U of vWF or 19.9 U/kg, and 1260 U or 93 U/kg, respectively) and a hemophilia A/vWD dog (36 U or 4.6 U/kg). In the two vWD dogs, plasma vWF activity increased from less than 1% to a median of 103% (Table 1). The plasma vWF:Ag increased from less than 1% to a median of 41.5%. The saline bleeding time (SBT) was corrected in both vWD dogs from more than 15 to 4.1 minutes. In the hemophilia A/vWD dog, the vWF antigen and activity increased to 15% and 44%, respectively, after infusion. The prolonged bleeding time was not corrected in this double-deficient dog. One additional vWD dog was given fresh normal canine PPP (850 U vWF or 32 U/kg). Although vWF activity levels of 54% and vWF:Ag of 67% were measured, bleeding time was only partially corrected to 12 minutes. A full range of multimers of vWF:Ag appeared in the plasma of these vWD and hemophilia A/vWD dogs after infusion of the FVIII-deficient cryoprecipitate and normal plasma. The distribution of high molecular weight multimers was comparable by laser densitometry to that present in normal canine pool plasma and FVIII-deficient cryoprecipitate infused (data not shown). Despite the presence of vWF functionally and antigenically, none of eight arteries (4 coronary, 4 carotid) in these four dogs developed evidence of thrombosis following stenosis and injury (Table 1).

Morphometry of stenosis and injury among phenotypes. The degree of stenoses induced by the Goldblatt clamp was similar when phenotypic groups were compared (data not shown). Most arteries were occluded to 80% or more of the cross-sectional area of the artery. Also, each group had one or more arteries with less stenosis. The degree of arterial injury as assessed by the amount of smooth muscle cell damage and disruption of the internal elastic lamina varied over a wide range but was similar across the phenotypic groups.

Hemodynamic values, hematocrit, and platelet counts. No significant differences were found in systolic blood pressure, heart rate, or double product (systolic blood pressure X heart rate) when normal, vWD, hemophilia A, and hemophilia A/vWD dogs were compared (data not shown). The diastolic pressure tended to be somewhat lower in the vWD dogs (normal = 69 ± 4.9, hemophilia A = 74 ± 4.1, vWD = 56 ± 6.8, P < .05, Kruskal-Wallis). Other investigators have shown that cyclic flow reduction in dogs continue unabated by lowering systolic blood pressure from 122.5 to 75.0 mm Hg. By necessity, a profound reduction in diastolic blood pressure would have also occurred. Given this finding, it seems unlikely that the lower diastolic blood pressure noted in our vWD dogs would explain the difference in the incidence of thrombosis. There were no significant differences between groups in hematocrits or platelet counts (Table 1).

DISCUSSION

The roles of plasma and platelet vWF and factor VIII were studied in dogs with a stenosis and injury model of induced arterial thrombosis. Occlusive thrombosis was consistently induced in normal and hemophilia A dogs, but vWD dogs did not develop occlusive thrombi even with infused canine vWF products present in the plasma. Platelet vWF was not detected in the dogs of any of the four phenotypes included in this study despite the presence of normal platelet α-granule fibronectin and normal platelet α-granule morphology (not shown). In this model, thrombosis fails to occur in the absence of vWF and is not supported by factor VIII deficient cryoprecipitate. Thus, replacement of plasma vWF does not support thrombosis as seen in dogs with vWF in the plasma and subendothelium. These data support the hypothesis that the plasma and subendothelium compartments of vWF support thrombosis independent of platelet vWF or of its association with FVIII.
Subjects with hemophilia A lack FVIII and have serious impairment of thrombin generation. Because human arterial thrombi are rich in fibrin in addition to platelets, arterial thrombosis occurring in the presence of vWF without FVIII may seem paradoxical. However, it has been observed that human patients with severe hemophilia A are not protected from myocardial infarction or thrombosis complicating aortic atherosclerosis. In both humans and dogs, then, vWF supports arterial thrombosis independent of its association with FVIII.

The mechanism by which vWF supports arterial thrombosis in dogs may involve vWF in two compartments: plasma vWF and exposed subendothelial matrix vWF. This hypothesis is supported by the finding that infused plasma vWF fails to support thrombosis in the vWD dogs with vWF-deficient subendothelial matrix (Table 1). Infusion of FVIII-deficient cryoprecipitate into vWD dogs provided plasma vWF with a full range of multimers that was readily detectable functionally and antigenically but would not have replaced subendothelial vWF. In dogs, then, vWF in the subendothelial matrix may be needed to support thrombosis in addition to plasma vWF. Studies in vWD pigs have also shown that plasma vWF alone is insufficient to support arterial thrombosis, whereas the combination of plasma and platelet vWF does support thrombosis. In vWD pigs, then, platelet vWF may interact with plasma vWF in the formation of...
thrombosis at sites of arterial stenosis and injury in the absence of subendothelial matrix vWF. Plasma vWF is the common denominator in a two-compartment model of vWF-dependent arterial thrombosis in these two vWF-deficient species: plasma and subendothelial matrix vWF in vWD dogs and plasma and platelet vWF in vWD pigs. Considered together, these data support the hypothesis that plasma vWF may activate, be activated by, or function as a cofactor to vWF in another compartment to support arterial thrombosis.

Bleeding time shortening following vWF infusion appears to differ among vWF-deficient species. Infusion of vWF concentrates largely corrected bleeding times in vWD dogs in this study but has failed to do so in vWD pigs. This difference in bleeding time response to vWF infusion between vWD dogs and pigs was observed even with plasma vWF activity levels greater than 100% in both species. In humans with vWD, a divergence also has been noted between bleeding time and vWF activity following vWF infusion. It appears in humans with type III (severe homozygous) vWD that replacement of plasma and platelet vWF are required to normalize the bleeding time, whereas plasma vWF alone is insufficient. Our current study in dogs considered with those performed in pigs and humans suggests there may be species differences in the bleeding time shortening in vWF-deficient subjects.

Several possibilities could explain these species differences in bleeding time shortening. Clearly, the variability in vWF compartmentalization among dogs, pigs, and humans could affect this test. It also is possible that plasma vWF in dogs corrects prolonged bleeding time by a mechanism that differs from that by which it supports thrombosis. This hypothesis is supported by the observation that infusion of vWF supports vWF-dependent bleeding time correction without supporting vWF-dependent arterial thrombosis. Alternatively, cryoprecipitation may concentrate other plasma constituents that could shorten the bleeding time in dogs independent of vWF. In addition, there may be changes in the molecular conformation of vWF induced by plasma harvesting or cryoprecipitation that would shorten bleeding time but not support arterial thrombosis. This hypothesis is supported by another report from our laboratory that documented a difference in the half-life of FVIII complexed with endogenous and exogenous vWF. Regardless of the mechanism, bleeding time shortening by plasma vWF in the absence of platelet or subendothelial matrix vWF in vWD dogs suggests that plasma vWF is the main determinant of the bleeding time in this species.

Other investigators have noted that occasionally mongrel dogs fail to develop thrombosis in this stenosis and injury model. Three such dogs had a vWF antigen of 92% ± 11%; however, the administration of deamino-8-D-arginine vasopressin (DDAVP), produced a rise in vWF antigen to 133% ± 12% that was accompanied by cyclic flow reductions. No other data on vWF were given in that abstract.

Other studies that have examined canine platelets for vWF antigen have yielded variable results, both the presence and absence of platelet vWF. Platelet vWF activity was not reported in these studies. In the present study, we used several physical and chemical procedures to isolate platelet vWF from PRP in several anticoagulant regimens. These procedures were successfully used to isolate porcine and human platelet vWF. For this study, we developed a rabbit anti-canine vWF antibody that was raised against vWF from a dog with hemophilia A and rendered monospecific by adsorption with canine vWD cryoprecipitate. This antibody readily detected vWF antigen in canine plasma but not platelet lysates. These findings are in agreement with those of Potter et al, who used rabbit anti-canine vWF antibodies. There are two reports that identified small amounts of vWF antigen in the platelets of mongrel dogs. In the first study, anti-human and anti-canine antibodies were used that had been rendered monospecific by adsorption with human but not canine vWD plasma. This anti-human vWF antibody was shown to be monospecific for human but not canine vWF by a rocket inhibition method against commercial anti-human vWF antisera. In the second study, an anti-human vWF antibody from a commercial source was used. No antibody characterization data were given in that study. It is possible that these anti-human vWF antibodies that were not rendered monospecific by adsorption with canine plasma or cryoprecipitate may have recognized epitopes other than canine vWF in canine platelets. It may also be that some breeds of dogs fail to express vWF in their platelets, whereas others express small amounts. We used dogs primarily from the closed colony of inbred dogs, thus providing homogeneous groups of normal, hemophilia A, and vWD. The source of normal canine platelets was mongrel dogs in the studies that detected platelet vWF antigen. Dogs, like humans, may have discordance in the expression of platelet and plasma vWF.

Halothane was used by itself for anesthesia in this study. This agent has been successfully used in studies of vWF in arterial thrombosis. Other investigators have shown that halothane in combination with thiamy] interrupted cyclic thrombosis in this model. It may be that the antiplatelet effect of barbiturates on platelets accounts for the apparent discrepancy between our report and that of Bertha et al. Our findings corroborate those of Barr et al, who found that halothane did not induce an antiplatelet effect in dogs.

Our study has shown that when vWF is absent, arterial thrombosis does not occur in this model. An interesting alternative hypothesis for the protection from thrombosis afforded by vWD in dogs could be a concomitant lack of other endothelial cell products such as tissue plasminogen activator. Such a combination of defects has been documented in humans. To confirm such a possibility would require characterization of the plasminogen activator response in our vWD dogs. This possibility will require further investigation.

vWF supports platelet adhesion, platelet spreading, and arterial thrombosis in several species. The contribution of vWF within plasma, endothelial cells, subendothelial matrix, and platelets to each of these processes is complex. The difference in expression of vWF by canine and porcine endothelium and platelets suggests that these two animal species can be used to explore differences in the function of vWF in its various compartments. Also, agents designed to prevent or treat thrombosis or hemorrhage by modifying vWF in plasma or platelets could be tested for differential results in these two species. Additional comparative studies in dogs,
pigs, and humans should continue to clarify the relative contribution of von Willebrand factor in its different compartments to thrombosis and hemostasis.

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