Effect of desmopressin on von Willebrand factor multimers in Doberman Pinschers with type 1 von Willebrand disease

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**Objective**—To assess the effect of desmopressin (DDAVP) administration in Doberman Pinschers with type 1 von Willebrand disease (vWD) on plasma von Willebrand factor (vWF) multimers through determination of vWF collagen binding activity (vWF:CBA; a functional vWF assay dependent on the presence of high–molecular-weight [HMW] multimers), comparison of vWF antigen concentration (vWF:Ag) to vWF:CBA, and vWF multimer size distribution.

**Animals**—16 Doberman Pinschers with type 1 vWD and 5 clinically normal control dogs.

**Procedure**—Plasma vWF:Ag and vWF:CBA assays and vWF multimer analysis were performed before and 1 hour after administration of DDAVP (1 µg/kg, SC).

**Results**—Following DDAVP administration, dogs with type 1 vWD had an increase in mean baseline values of plasma vWF:Ag and vWF:CBA from 10% to 17% for both variables. The mean vWF Ag:CBA ratio at baseline (0.95) was similar after DDAVP administration (0.97), indicating concordant increases in plasma vWF concentration and activity. In control dogs, mean plasma vWF:Ag and vWF:CBA increased from baseline values of 64% to 113% and 58% to 114%, respectively, and the vWF Ag:CBA ratios were unchanged (1.1 vs 1.0) after DDAVP administration. Plasma vWF multimer analysis revealed proportional increases in band intensity for all multimer sizes following DDAVP administration, in comparison to baseline for the control dogs and Doberman Pinschers with vWD, consistent with vWF Ag:CBA ratios of approximately 1.

**Conclusions and Clinical Relevance**—Beneficial effects of DDAVP on primary hemostasis in Doberman Pinschers with type 1 vWD cannot be explained by preferential increases in HMW vWF multimers. (Am J Vet Res 2005;66:861–867)

**Von Willebrand factor** (vWF), a large glycoprotein synthesized by and stored in endothelial cells, has a multimeric structure consisting of approximately 250-kd subunits connected by disulfide bonds and ranging in molecular weight size from 500 kd (dimer) to > 20,000 kd. High–molecular-weight (HMW) vWF multimers, defined as having a molecular mass ≥ 6,000 kd, may be more hemostatically effective by supporting, to a greater degree, platelet adhesion to the vascular subendothelium and inducing platelet aggregation under conditions of high shear forces.3,7 **Von Willebrand disease** (vWD) is the most common mild hereditary bleeding disorder in humans and dogs and is classified on the basis of plasma vWF concentration and multimeric structure into 3 types.5 Type 1 vWD is defined by a low plasma vWF concentration (< 50% of reference range values) with a normal multimer distribution, whereas type 2 vWD is defined by a low plasma vWF concentration with a disproportionate decrease in the HMW vWF multimers. In type 3 vWD, plasma vWF is virtually undetectable (< 1%). Type 1 vWD is by far the most common form, having been reported in > 70 breeds and affecting more than half of Doberman Pinschers. The most widely used screening test for canine vWD is an ELISA-based vWF antigen concentration (vWF:Ag) assay, a quantitative measure of plasma vWF concentration. The vWF collagen binding activity (vWF:CBA) assay, also an ELISA, is a functional vWF assay that is dependent on the presence of HMW vWF multimers and is therefore helpful in determining hemostatic efficacy and differentiating type 2 from type 1 vWD.14 In addition, mutation-specific tests have become available for screening in a few breeds of dogs, including the Scottish Terrier, Dutch Kooiker, Shetland Sheepdog, Doberman Pinscher, Bernese Mountain Dog, and Pembroke Welsh Corgi.1a,7

**Desmopressin (DDAVP),** a synthetic analogue of the neurohypophyseal hormone arginine vasopressin, has been used to control bleeding in a variety of hemostatic disorders but most commonly in vWD in humans and dogs. Although the beneficial hemostatic effects of DDAVP in humans with vWD and hemophilia A have been mainly attributed to increases in plasma concentrations of vWF and factor VIII, respectively, it is recognized that DDAVP is efficacious in hereditary and acquired thrombopathies in human patients who have plasma concentrations within the reference range or even high concentrations of vWF and factor VIII, such as in patients with uremia.5,14 It has been proposed that favorable hemostatic effects of DDAVP may be mediated in part by the new appearance of ultralarge vWF multimers in plasma.7 A disproportionate increase in HMW vWF multimers and a greater increase in vWF:CBA relative to vWF:Ag have been found in the plasma of humans with type 1 vWD following administration of DDAVP.13
The purpose of the study reported here was to assess the effect of DDAVP administration in Doberman Pinschers with type 1 vWD on plasma vWF multimers through determination of vWF:CBA, comparison of vWF:Ag to vWF:CBA, and vWF multimer size distribution before and 1 hour after treatment.

Materials and Methods

Animals—Blood was collected from 16 client-owned Doberman Pinschers with type 1 vWD. Diagnoses of type 1 vWD were made on the basis of plasma vWF concentrations of < 35% of a control pooled plasma from clinically normal dogs, as determined by use of a validated ELISA.16 Dogs with vWD ranged in age from 6 months to 11 years and included 8 males (2 sexually intact, 6 castrated) and 8 females (2 sexually intact, 6 spayed). None of the dogs received blood component treatment or DDAVP during the month prior to this study. Fourteen of the 16 dogs with vWD were previously described in a report17 on the effect of DDAVP administration on primary hemostasis as assessed by a point-of-care instrument.1 Blood was also collected from 5 clinically normal mixed-breed dogs (4 sexually intact females and 1 sexually intact male) from a research colony at the University of Pennsylvania School of Veterinary Medicine. None of the female dogs were near estrus or pregnant during the study. The Institutional Animal Care and Use Committee at the University of Pennsylvania School of Veterinary Medicine and the Committee for the Use of Client Owned Animals in Research approved the study protocol; informed owner consent was obtained.

Blood sample collection and DDAVP testing—All blood samples (4.5 mL) were obtained with minimum trauma via cephalic, saphenous, or jugular venipuncture (20-gauge needle) and collected directly into evacuated tubes containing one tenth the volume of 3.8% sodium citrate as the anticoagulant.7 After obtaining citrated blood samples for measurement of baseline plasma vWF:Ag and vWF:CBA and vWF multimer analysis, DDAVP was administered SC at a dose of 1 µg/kg was administered SC.18,19 and citrated blood samples were collected 1 hour later for post-treatment testing. The blood was chilled and centrifuged (1,000 × g for 5 minutes), and the plasma was separated and frozen in aliquots at −70°C. For 2 dogs with vWD and 2 control dogs, blood was additionally collected at 15, 30, and 45 minutes after DDAVP administration for determination of the same variables.

vWF testing—Plasma vWF:Ag and vWF:CBA were assayed by ELISAs, as previously described.1 The vWF:Ag and vWF:CBA assays have a coefficient of variation of 3.8% and 4.4%, respectively. The ELISA used to determine vWF:CBA was modified to use commercially available mixed type I/III bovine collagen as the capture substrate and a murine monoclonal antibody to canine vWF to report vWF binding to immobilized collagen. Plasma vWF multimers were separated by a stacked SDS-agarose gel electrophoresis in a modification of the method of Ruggeri and Zimmerman.10 Briefly, the running and stacking gels contained 1.4% and 0.7% agarose (high-gelling-temperature agarose for resolution of HMW vWF multimers), respectively. The running-gel buffer was 0.081M Tris, 0.024M Tricine, 0.0004M calcium lactate, (pH, 8.5), and the stacking-gel buffer was 0.125M Tris-HCl, (pH, 6.8). Plasma samples from clinically normal and vWD-affected dogs were diluted 1:5 and 1:3, respectively, in sample buffer (0.1M Tris, 0.1M NaEDTA, 8.0M urea, and 2% SDS, (pH, 7.0)), and 15 µL of diluted sample was applied to each well in the stacking gel. In addition, plasma samples from each dog were adjusted to the same vWF:CBA as the baseline activity to improve monitoring for the appearance of HMW vWF multimers. Samples were run at 60 V until the tracking dye reached the end of the gel (approx 9 hours). Plasma vWF multimers were electrophoretically transferred (80 V for 12 hours) to nitrocellulose membranes by use of plate electrodes and a cooling coil attached to a recirculating cooling bath set at 10°C to maintain a gel temperature of 15° to 18°C. The transfer buffer was 0.025M Tris, 0.193M glycine, 20% methanol, and 0.1% SDS, (pH, 8.3). Nitrocellulose membranes were blocked, incubated with the primary antibody (rabbit anti-human vWF), washed, and then incubated with the secondary antibody (horseradish peroxidase-labeled goat anti-rabbit IgG). The vWF multimers were then detected by chemiluminescence.1

Statistical analyses—Differences in plasma vWF:Ag, vWF:CBA, and vWF Ag:CBA before and after DDAVP administration were determined by a repeated-measures ANOVA. Results were considered significant at P < 0.05.

Results

Doberman Pinschers (n = 16) with vWD had baseline plasma vWF:Ag and vWF:CBA values ranging from 4% to 19% (mean ± SD, 10 ± 4.9%) and 6% to 19% (10 ± 3.9%), respectively.7 The median vWF:Ag:CBA ratio at baseline was 0.95 ± 0.23, reflecting a proportional amount of vWF quantity to activity. One hour following DDAVP administration, a significant (P < 0.001) increase was found in plasma vWF:Ag, with values ranging from 8% to 38% (17 ± 9%), but only 1 dog reached a value > 35%. Similarly, a significant (P = 0.001) increase was found in vWF:CBA after DDAVP administration, with values ranging from 10% to 34% (17 ± 6.4%; Figure 1). The mean vWF:Ag:CBA ratio following DDAVP administration was 0.97 ± 0.25, indicating a concordant increase in vWF:Ag and vWF:CBA resulting from an increase in the plasma concentration of the full range of vWF multimers. In comparing vWF:CBA before and after administration of DDAVP (pretreatment values to post-treatment values), the mean ratio was 0.63 ± 0.21, indicating an increase in the functional activity of vWF as a result of an increase in the HMW vWF multimers that are selectively captured in the vWF:CBA assay.

Control dogs had mean baseline plasma vWF:Ag, vWF:CBA, and vWF Ag:CBA of 64 ± 13.1%, 58 ± 8.8%, and 1.1 ± 0.11, respectively. One hour following administration of DDAVP, a significant (P < 0.001) increase in mean plasma vWF:Ag (113 ± 14.6%) and vWF:CBA (114 ± 13.9%) was found, with a mean vWF:Ag:CBA ratio of 1.0 ± 0.1 (Figure 1). In comparing vWF:CBA before and after administration of DDAVP (pretreatment values to post-treatment values), the mean ratio was 0.52 ± 0.06. Failure to demonstrate a selective increase in only HMW vWF multimers or the new appearance of ultralarge multimers 1 hour after DDAVP administration could be attributed to rapid clearance of vWF multimers from plasma or cleavage into smaller multimers before sample collection at 1 hour. Therefore, to document that the appearance of HMW vWF multimers was not missed, blood samples were obtained at baseline and at 15, 30, 45, and 60 minutes after DDAVP administration in 2 Doberman Pinschers and 2 clinically normal control dogs. Increases in plasma vWF:Ag and vWF:CBA were observed as early as 15 minutes.
and gradually increased further through 60 minutes in the control dogs; however, changes in plasma vWF:Ag and vWF:CBA were negligible for the 2 Doberman Pinschers with vWD throughout the time course (Table 1). The vWF:Ag:CBA ratios were relatively constant from baseline through 60 minutes, supporting an increase in the plasma concentration of the full range of vWF multimers from 15 to 60 minutes following DDAVP administration.

Plasma vWF multimer analysis revealed proportional increases in band intensity for all multimer sizes following DDAVP administration, in comparison to baseline samples from control dogs and Doberman Pinschers with vWD, consistent with the mean vWF:Ag:CBA ratios of approximately 1 after DDAVP administration (Figure 2). Plasma vWF multimer analysis performed by use of samples adjusted to the same vWF:CBA revealed almost identical band intensity for all multimer sizes at every time point following DDAVP administration, in comparison to baseline samples from control dogs and Doberman Pinschers with vWD, further demonstrating that dogs do not respond to DDAVP with a preferential increase in HMW vWF multimers.

Figure 1—Mean ± SE changes in plasma von Willebrand factor (vWF) variables in Doberman Pinschers (n = 16) with type 1 von Willebrand disease (vWD) and control dogs (5) before (Pre) and 1 hour after (Post) administration of desmopressin (DDAVP). A—Plasma vWF antigen concentration (vWF:Ag). B—Plasma vWF collagen binding activity (vWF:CBA). *Significantly (P ≤ 0.001) different from baseline or values before DDAVP administration.

Table 1—Time course for changes in von Willebrand factor (vWF) antigen concentration and collagen binding activity in 2 Doberman Pinschers with type 1 von Willebrand disease (vWD) and 2 control dogs following desmopressin administration.

<table>
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vWF:Ag = von Willebrand factor antigen concentration. vWF:CBA = von Willebrand factor collagen binding activity. vWF:Ag:CBA = Ratio of vWF:Ag to vWF:CBA.
Discussion

Species differences have previously been recognized regarding the hemostatic response to DDAVP administration. In humans, administration of DDAVP results in a 2- to 5-fold increase in plasma vWF concentration in clinically normal individuals as well as those with type 1 vWD, whereas the effect of DDAVP on plasma vWF concentration in clinically normal dogs and those with type 1 vWD is much less dramatic with an approximate 25% to 70% increase above baseline in some but not all dogs tested. Despite this modest increase in plasma vWF concentration, administration of DDAVP to Doberman Pinschers with type 1 vWD has resulted in improved hemostatic function as assessed by a point-of-care primary hemostasis function analyzer, and, most importantly, control of hemorrhage and less surgical bleeding in Doberman Pinschers with vWD.

Results of our study document another difference between humans and dogs regarding response to DDAVP administration. Although a greater increase in plasma vWF:CBA than vWF:Ag has been observed in humans following administration of DDAVP as a result of a disproportionate increase in HMW vWF multimers, a concordant increase in quantity and functional activity of vWF as a result of a proportional increase in all vWF multimer sizes has been observed in dogs. Results of an early study (predating the new classification system for vWD approved by the International Society on Thrombosis and Haemostasis Subcommittee on vWD in 1994 and the more recent knowledge of gene defects and different mechanisms underlying type 2A vWD) evaluating multimeric composition of vWF following administration of DDAVP to humans with different subtypes of vWD and clinically normal individuals suggested that platelets may be the source for the largest vWF multimers released. In clinically normal individuals and those with type 1 vWD, the plasma concentration of all vWF multimers present under basal conditions increased in response to DDAVP administration; however, larger multimers than were initially present also appeared in the plasma, reaching a maximum at 1 hour. These ultralarge multimers were similar in size to those found in platelets of clinically normal individuals and those with type 1 vWD and disappeared from circulation by 4 hours. In humans with type 2A vWD, in which large vWF multimers are missing from the plasma but present in platelets, DDAVP administration produced a dramatic alteration in plasma vWF multimeric composition with the appearance of larger multimers in the plasma within 15 minutes, leading to a multimeric composition that was qualitatively not different from clinically normal individuals.

Figure 2—Plasma vWF multimer gel electrophoretic analyses from 2 control dogs and 2 Doberman Pinschers with type 1 vWD before (time 0) and after administration of DDAVP (1 µg/kg, SC). Plasma samples were diluted 1:5 and 1:3 with sample buffer for the control dog and Doberman Pinscher with vWD, respectively. For the control dog, baseline vWF:Ag and vWF:CBA were 73% and 62%, respectively, and 1 hour after DDAVP administration 135% and 119%, respectively, for the dog with vWD, baseline vWF:Ag and vWF:CBA were 8% and 11%, respectively, and 1 hour after DDAVP administration 10% and 11%, respectively. For the time course (evaluations at baseline and 15, 30, 45, and 60 minutes following DDAVP administration), plasma samples were adjusted to the same vWF:CBA as baseline for a control dog (vWF:CBA, 53%) and a Doberman Pinscher with vWD (vWF:CBA, 12%) to enhance monitoring for the appearance of high–molecular-weight (HMW) vWF multimers. The plasma vWF multimers increase in size from the bottom of the figure (low–molecular-weight (LMW)) toward the top (HMW).
However, the larger multimers disappeared from the plasma rapidly, with some clearing evident at 60 minutes. Although Ruggeri et al. did not conclusively determine that larger multimers appearing in plasma following DDAVP administration originated from platelets, it is interesting to speculate that the lack of a similar finding in clinically normal dogs and those with type 1 vWD may be associated with the negligible amounts of vWF in normal canine platelets, unlike normal human platelets. In addition, in a study describing a measurable but negligible amount of vWF in canine platelets (2% of circulating vWF with 15% of this being released into the fluid phase on in vitro activation), it was observed that canine platelets did not have increased expression of the largest multimers reported for human platelet vWF.

In a recent study evaluating changes in vWF multimer size following DDAVP administration in humans with type 2A vWD, a difference was observed in response depending on the genetic mutation responsible for the disorder. In patients with mutations causing defective intracellular transport and assembly of vWF no change was found in the proportion of HMW vWF multimers after DDAVP administration nor was there an improvement in bleeding time or increase in vWF function as assessed by the vWF ristocetin cofactor assay. However, in patients with mutations resulting in increased proteolysis of vWF multimers after secretion into the plasma, a transient appearance of HMW vWF multimers was observed after DDAVP administration, in conjunction with a transient normalization of the bleeding time and improvement in vWF ristocetin cofactor activity.

Rapid clearance from the plasma of the larger vWF multimers induced by DDAVP administration, as observed in some humans with type 2A vWD, or cleavage of HMW vWF multimers to smaller forms are potential explanations for the failure to observe the new appearance of larger vWF multimers in clinically normal dogs and Doberman Pinschers with type 1 vWD. Plasma vWF:Ag and vWF:CBA peaked at 30 to 60 minutes and remained stable during this first hour; the new appearance of larger vWF multimers was not observed at any time point, suggesting that rapid clearance or cleavage of HMW vWF multimers is not a likely mechanism accounting for the absence of new, larger vWF multimers at 60 minutes.

Given that vWF multimer analysis is a technically difficult and time-consuming assay and functional vWF assays, such as ristocetin or botrocetin cofactor assays, are difficult to standardize and not highly sensitive, there has been much interest in use of the vWF:CBA assay. vWF assays, such as ristocetin or botrocetin cofactor activity, are difficult to standardize and not highly sensitive, indicating that this vWF:CBA assay is sensitive to a decrease or absence of the more functionally active HMW vWF multimers.

A study investigating the use of a vWF:CBA assay for monitoring responsiveness of humans with type 1 vWD to DDAVP treatment documented a consistently greater increase in vWF:CBA (6- to >25-fold) than vWF:Ag (2- to 5-fold) following DDAVP administration, compared with baseline, attributed to a disproportionate increase in the more functionally active HMW vWF multimers. Interestingly, the standard vWF functional assay in humans, the vWF ristocetin cofactor assay, appeared to be poorly sensitive in assessing responsiveness to DDAVP. In our study, the concordant increase in vWF:Ag and vWF:CBA following DDAVP administration to clinically normal dogs and Doberman Pinschers with type 1 vWD (ie, a vWF Ag:CBA ratio of approx 1 before and after DDAVP administration) indicated that a proportional increase in all vWF multimer sizes occurs rather than a disproportionate increase in HMW vWF multimers as observed in humans. Although the use of the vWF:CBA assay, in addition to the vWF:Ag assay, has been proposed for a potentially more accurate estimation of a human patient’s functional responsiveness to DDAVP in dogs the vWF:CBA assay does not offer this advantage because vWF:Ag and vWF:CBA change in parallel following DDAVP administration.

Despite the distinct differences in response to DDAVP between humans and dogs as assessed by plasma vWF:Ag, vWF:CBA, and results of vWF multimer analysis, subjectively it appears that DDAVP administration leads to improved clinical control of surgical or spontaneous bleeding in many Doberman Pinschers with type 1 vWD. This improvement may in part be associated with post-DDAVP increases in those molecular weight forms of canine vWF ranging from >6,000 to 20,000 kd that normally circulate in plasma and have enhanced hemostatic activity relative to multimers of lower molecular size. Although it has been proposed that the favorable hemostatic effects of DDAVP observed in humans may be mediated in part by the new appearance in plasma of ultralarge (>20,000 kd) vWF multimers, this mechanism is not responsible for the improvement in primary hemostasis found in Doberman Pinschers with type 1 vWD following DDAVP administration. However, 2 previous studies evaluating the effects of DDAVP administration on plasma vWF multimers and vWF botrocetin cofactor activity as a functional vWF assay in beagle dogs and Doberman Pinschers with type 1 vWD came to a different conclusion (ie, increases in functional vWF activity are greater than increases in vWF:Ag as a result...
of preferential increases in the HMW vWF multimers). Reasons for the discrepancy are not clear, although differences in technique for the vWF multimer analysis may have made it more difficult to evaluate HMW vWF multimers in the earlier studies, given that the authors acknowledge that only 9 bands could be clearly distinguished, as opposed to the goal of achieving at least 15 discrete bands to better evaluate HMW multimers. Functional assays, such as the vWF ristocetin cofactor assay used for human vWF and the vWF botrocetin cofactor assay used for canine vWF, are semiquantitative tests of vWF-dependent platelet agglutination in which a comparison is made between the agglutination response of normal platelets combined with an agglutinating reagent (either ristocetin or botrocetin) and either patient plasma or dilutions of control plasma as a source of vWF. These cofactor assays are difficult to standardize, and comparison of the vWF ristocetin cofactor assay with the vWF:CBA assay in evaluating responses of humans with type 1 vWD to DDAVP revealed that the vWF:CBA assay was not sensitive. The vWF botrocetin cofactor activity was not evaluated in our study to determine how it compares to the vWF:CBA, but the ELISA used for determination of vWF:CBA was easily standardized and appeared sensitive to the presence of the HMW vWF multimers, as evidenced by the decrease in vWF:CBA observed in dogs with type 2 vWD.

Recently, Olsen et al. attempted to assess the effect of repeated large doses of DDAVP (5 µg/kg, IV, for 7 days) in 2 clinically normal dogs and 1 mixed-breed dog heterozygous for type 3 vWD. They documented a similarly modest increase in plasma vWF:Ag (60% increase over baseline for the dog with vWD) in these dogs, with tachyphylaxis following repeated administration of DDAVP. Plasma vWF multimer analyses for 2 control dogs did not permit clear identification of bands corresponding to HMW vWF multimers or convincingly demonstrate an increase in HMW vWF multimers at any time point following DDAVP administration, and an immunoblot was not provided for the 1 dog with vWD.

Although > 20 years of clinical experience with DDAVP administration in humans has more firmly established the clinical indications for this medication, the mechanisms of action are still not completely understood. The mechanisms for improved hemostasis and the reasons for differences in response between humans and dogs to DDAVP administration were not addressed in our study. However, the concordant increase in vWF quantity (vWF:Ag) and functional activity (vWF:CBA) and proportional increases in band intensity for all multimer sizes in plasma samples following DDAVP administration, in comparison to baseline samples from control dogs and Doberman Pinschers with type 1 vWD, indicate that the beneficial effect of DDAVP on primary hemostasis in dogs cannot be explained by a preferential increase in HMW vWF multimers, as has been suggested in humans. The data of our study suggest that the storage pool of vWF multimers in dogs may differ from that of humans and other species (eg, mice) in that canine vWF may be packaged in storage granules in a size range similar to that in plasma, in which case ultralarge vWF multimers are not available for release. Alternatively, if such large multimers are released, they may remain tightly bound to components of the damaged subendothelium and not be detected in the plasma following DDAVP administration. Future studies with cultured canine endothelial cells to evaluate vWF packaging and release may increase our understanding of species differences in response to DDAVP.

References


