The Effects of Desmopressin on Plasma Factor VIII/ von Willebrand Factor Activity in Dogs with von Willebrand's Disease

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ABSTRACT

Eight unanesthetized normal dogs and seven dogs with von Willebrand's disease (vWD) were given desmopressin (0.6 μg/kg, IV) in order to determine the effects of this drug on plasma Factor VIII/vWF activity. Seven of the normal dogs and four of the vWD dogs were administered an equal volume of saline (control infusion) on another occasion. The other three vWD dogs underwent major surgery after treatment with desmopressin. Plasma FVIII coagulant activity (FVIII:C), von Willebrand factor antigen (vWF:Ag), and FVIII-ristocetin cofactor activity (FVIII:RC) were quantitated before infusion and at 60 minutes postinfusion. Activities were expressed as a percentage of the activity of a pooled canine plasma (12 dogs) arbitrarily designated as having 100% FVIII:C, vWF:Ag, and FVIII:RC activity.

Plasma FVIII:C activity increased by 28% in the normal dogs and by 37% in the dogs with vWD. Plasma vWF:Ag increased more than twofold in normal dogs after desmopressin treatment. In the vWD dogs the average increase was also twofold, however there was much greater variability between dogs with increases ranging from 1.2 fold to 2.4 fold. Plasma FVIII:RC activity almost doubled in normal dogs, however like vWF:Ag, the increases in vWD dogs were more variable. One vWD dog had no increase in FVIII:RC while in the remaining six dogs FVIII:RC increases ranged from 1.8 to 2.9 fold.

The results of this study indicate that a single intravenous dose of desmopressin (0.6 μg/kg) causes a significant elevation in plasma vWF:Ag and FVIII:RC activity and a much lesser increase in FVIII:C activity in normal unanesthetized dogs. A similar but more variable response is seen in dogs with vWD. It is suggested that desmopressin may be useful in partially correcting the FVIII/vWF deficiency in some dogs with vWD and in improving in vivo hemostasis.

Key words: Canine, desmopressin, von Willebrand's disease, Factor VIII/von Willebrand factor complex.

RÉSUMÉ

Cette expérience portait sur huit chiens normaux et sur sept autres, atteints de la maladie de von Willebrand, non anesthésiés. Elle consistait à leur administrer une injection intraveineuse de 0,6 μg/kg de desmopressine, afin d'en déterminer les effets sur le complexe facteur plasmatique VIII — facteur de von Willebrand. Sept des chiens normaux et quatre de ceux qui souffraient de la maladie précitée subirent une intervention chirurgicale majeure, après l'administration de desmopressine. Les auteurs quantifièrent l'activité coagulante du facteur VIII, celle du facteur antigénique de von Willebrand et celle du complexe facteur VIII — cofacteur ristocétine, avant l'injection de desmopressine et 60 minutes après. Ils exprimèrent ces trois activités respectives sous la forme d'un pourcentage de celles d'un pool du plasma de 12 chiens normaux auxquelles ils attribuèrent arbitrairement la valeur de 100%.

L'activité coagulante du facteur VIII augmenta de 28%, chez les chiens normaux, et de 37%, chez ceux qui souffraient de la maladie précitée. L'antigène du facteur de von Willebrand fit plus que doubler chez les chiens normaux, après l'injection de desmopressine; il afficha une augmentation comparable chez ceux qui étaient atteints de la maladie précitée, tout en manifestant plus de variations individuelles qui allaient de 1,2 à 2,4 fois la normale. L'activité du complexe facteur VIII — cofacteur ristocétine doubla aussi chez les chiens normaux mais, comme celle du facteur de von Willebrand, elle présenta plus de variations chez les chiens atteints de la maladie précitée. L'un de ces derniers ne manifesta aucune augmentation de l'activité du complexe facteur VIII — cofacteur ristocétine, contrairement aux six autres où elle atteignit de 1,8 à 2,9 fois la normale.

Les résultats de cette expérience indiquent qu'une seule injection intraveineuse de 0,6 μg/kg de desmopressine provoque une augmentation appréciable de l'activité de l'antigène du facteur de von Willebrand et de celle du complexe facteur VIII-cofacteur ristocétine, mais une augmentation beaucoup moins significative de l'activité coagulante du facteur VIII, chez les chiens normaux non anesthésiés. On constate une réaction semblable, mais plus variable, chez ceux qui souffrent de la maladie de von Willebrand. Les auteurs sous-entendent que l'acétate de desmopressine aiderait à corriger partiellement la déficience du complexe facteur VIII — facteur de von

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Willebrand, chez certains chiens atteints de la dite maladie, et à améliorer l'hémostate *in vivo*.

Mots clés: Chiens, desmopressine, maladie de von Willebrand, complexe facteur VIII — facteur de von Willebrand.

INTRODUCTION

Von Willebrand's disease (vWD) is the most common inherited bleeding disorder in dogs, and occurs with particularly high frequency in breeds such as the Doberman pinscher (1,2,3). The increased bleeding tendency in clinically affected dogs is the reflection of an abnormality in Factor VIII/von Willebrand factor (FVIII:vWF), a major hemostatic protein.

Factor VIII/vWF circulates as a protein complex consisting of two noncovalently bound molecular components (4,5,6). The large component is von Willebrand factor (vWF) and is expressed immunologically as von Willebrand factor antigen (vWF:Ag), or FVIII-related antigen (FVIII R:Ag) as it was formerly known (6). The functional activity of vWF can be quantitated using the antibiotic ristocetin since the aggregation of platelets by this drug occurs only in the presence of vWF. This functional activity of vWF is called ristocetin cofactor (FVIII:RC). Von Willebrand is the part of the FVIII/ vWF complex that is involved as a cofactor in platelet adhesion (4,7). The small molecular weight component of the FVIII/vWF complex is a cofactor in blood coagulation and is expressed as FVIII coagulant (FVIII:C) activity (5).

Von Willebrand's disease is most frequently characterized by a quantitative and/or qualitative deficiency of vWF. Plasma FVIII:C activity may be normal or subnormal. The degree of deficiency, and clinical signs, vary from mild to very severe. Treatment of bleeding episodes or prophylactic treatment to prevent bleeding, is primarily aimed at normalizing the FVIII/vWF abnormality by blood (plasma) transfusion or other means (2,8,9).

The vasopressin-analog DDAVP (desmopressin; 1 desamine-8-d-arginine vasopressin) has been shown to induce significant elevations in plasma FVIII:C, vWF:Ag and FVIII:RC activities in normal people and in people with some types of FVIII/vWF deficiency states (10,11,12,13). These observations have resulted in DDAVP being recommended as an alternative to blood component therapy in the treatment of some forms of vWD and classical hemophilia in man (14). Studies in normal dogs have shown that DDAVP also stimulates elevations in plasma vWF:Ag and FVIII:C activity in this species, however higher doses appear to be required and the rise in FVIII:C is much less pronounced than in man (15).

The purpose of this study was to determine the effects of DDAVP on plasma FVIII/vWF activity in dogs with vWD in order to assess how effective the drug might be in normalizing FVIII/vWF activity in this common canine bleeding disorder.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The dogs used in this study consisted of eight clinically normal mature dogs of mixed breeding, and seven dogs of Doberman pinscher or Doberman pinscher-cross breeding diagnosed as having vWD. The diagnosis of vWD was based on a subnormal plasma vWF:Ag level (on at least three separate testings), and historical evidence of apparent increased bleeding tendency, and/or evidence of close relationship (pedigree evaluation) to other affected animals (1,2,9).

The normal dogs and four dogs with vWD were housed in the Central Animal Facility at the University of Guelph. The remaining three dogs with vWD were privately owned dogs which were previously identified through our vWD genetic screening program, and were made available by their owners for use in this study. The latter dogs were all historical "bleeders" and were being prepared for major surgery. Infusion into these dogs however was carried out prior to anesthesia and surgery.

DDAVP INFUSIONS AND BLOOD SAMPLING

Each dog was fasted overnight and then allowed to acclimatize to the testing locale for at least 15 minutes prior to infusion. The DDAVP (Desmopressin acetate, Richmond Pharmaceuticals, Toronto, Ontario) was administered intravenously at a dosage of 0.6 μg/kg body weight. The required volume of stock solution (4 μg/mL) was diluted in a total volume of 15 mL saline immediately before administration, then was given slowly over a 7 min interval.

A blood sample was collected by cephalic venipuncture immediately prior to DDAVP infusion (pre-infusion sample) and at 60 min following the onset of infusion (postinfusion sample). Previous studies on normal dogs had shown peak rises in vWF and FVIII:C activity to occur 30-60 min postinfusion (15). In each case, 9 mL of blood was drawn into a plastic syringe containing 1 mL of 3.8% trisodium citrate anticoagulant, and thoroughly mixed. The postinfusion sample was drawn from the contralateral leg vein.

Seven of the eight normal dogs were given a control infusion of 15 mL saline on an occasion at least two weeks distant from the DDAVP infusion. Four of the dogs with vWD received two infusions of DDAVP separated by at least two weeks; the results for the two infusions were averaged. Three vWD dogs were available for infusion on only one occasion; it was therefore only possible to give one DDAVP infusion and a saline (control) infuson could not be administered. The latter three dogs were anesthetized and underwent major surgery after the postinfusion blood sample was taken.

Platelet-poor plasma (PPP) was obtained by centrifugation of the citrated blood at 2500 x *g* and 4° C for 20 min in siliconized glass tubes. Aliquots of plasma were frozen to 70° C for storage until assayed (usually within two weeks of collection).

FACTOR VIII/vWF ASSAYS

Plasma FVIII:C activity was quantitated by a differential partial thromboplastin time assay (2,15). Plasma vWF:Ag was measured using an electroimmunoassay as described elsewhere (15). Plasma FVIII:RC was assayed using a modification of a previously described aggregometry technique in which human formalin-fixed platelets were aggregated by ristocetin (final concentration 1.5 mg/mL) in the presence of varying concentrations of canine plasma (7,16).

All FVIII/vWF activities were expressed as a percent of normal activity. The normal reference plasma, arbitrarily designated as having 100% FVIII:C, vWF:Ag and FVIII:RC activities, was prepared by pooling PPP obtained from ten clinically normal dogs.

STATISTICAL ANALYSIS

The results of this study were expressed as group mean ± standard error of the mean (X ± SEM). Statistical comparisons between group means were performed using the Student's 't' test with 'p' values of <0.05 being considered statistically significant.

RESULTS

In the normal dogs, plasma FVIII:C activity increased by 28% ($p<0.01$) following the intravenous infusion of DDAVP in saline at a dosage of 0.6 μg/kg body weight (Table I). Plasma vWF:Ag and FVIII:RC activity increased to a much greater extent in these normal dogs (128% and 80% increases respectively). The infusion of saline alone caused no significant change in any of the FVIII/vWF activities in normal dogs.

In the dogs with vWD, DDAVP consistantly produced a significant rise in plasma FVIII:C activity by 60 min postinfusion ($p<0.01$). Although the initial plasma FVIII:C activity in vWD dogs was significantly lower than that in normal dogs ($p<0.01$), the levels in these dogs increased by an average of 37% after DDAVP infusion (Table I). Both plasma vWF: Ag and FVIII:RC activity also tended to increase after DDAVP administration in dogs with vWD, however the increases were not statistically significant ($p=0.21$ and 0.20 respectively). This lack of statistical significance in the responses in dogs with vWD resulted from the great variability between dogs. Where two infusions were given to the same dog, however, the response was generally similar on both occasions (Table II). As can be seen from Table II, plasma vWF:Ag elevations in dogs with vWD ranged from only 20% to over 140%. Plasma FVIII:RC activity increased from 75% to 200% in six of the vWD dogs, however one dog (Dog #7) showed no increase in FVIII:RC activity after DDAVP. There was no significant change in any of the FVIII/vWF activities in the four dogs with vWD which were infused with saline.

TABLE I. Effects of DDAVP Infusions on Plasma Factor VIII/vWF Activity in Normal Dogs and Dogs with von Willebrand's Disease

Dogs	FVIII:C[a]		vWF:Ag[a]		FVIII:RC[a]	
	Pre	Post[b]	Pre	Post	Pre	Post
Normal						
DDAVP 0.6 μg/kg (n=8)	85 ± 5	109 ± 5[c]	87 ± 7	198 ± 17[c]	111 ± 8	200 ± 28[c]
Saline control (n=7)	83 ± 3	87 ± 2	90 ± 8	86 ± 11	97 ± 6	89 ± 3
vWD						
DDAVP 0.6 μg/kg (n=7)	63 ± 4	86 ± 6[c]	15 ± 4	30 ± 11	27 ± 9	68 ± 28
Saline control (n=4)	66 ± 5	70 ± 6	18 ± 7	17 ± 6	35 ± 12	32 ± 11

[a] Expressed as a percent of the pooled normal canine reference plasma (Mean ± SEM)
[b] 60 min post DDAVP infusion (or saline for control)
[c] Statistically significant difference between pre and postinfusion blood samples

TABLE II. Individual Responses of Dogs with von Willebrand's Disease to DDAVP Infusions (0.6 μg/kg)

Dog	FVIII:C[a]		vWF:Ag[a]		FVIII:RC[a]	
	Pre	Post[b]	Pre	Post	Pre	Post
#1[c]	52 ± 3	73 ± 5	15 ± 13	33 ± 2	19 ± 2	44 ± 0
#2[c]	65 ± 2	90 ± 0	13 ± 0	16 ± 0	15 ± 3	29 ± 5
#3[c]	77 ± 0	115 ± 9	35 ± 5	81 ± 14	54 ± 0	145 ± 14
#4[c]	72 ± 0	101 ± 0	23 ± 0	56 ± 0	70 ± 6	201 ± 29
#5[d]	55	68	5	8	7	21
#6[d]	67	78	9	13	16	28
#7[d]	51	75	5	6	11	7

[a] Expressed as a percent of the pooled normal canine reference plasma (Mean ± SEM)
[b] 60 min postinfusion
[c] Two DDAVP infusions
[d] Single DDAVP infusion

DISCUSSION

The results of this study have confirmed previous observations indicating that DDAVP given as a single intravenous injection at a dosage of 0.6 μg/kg, induces elevations in plasma FVIII/vWF activities in unanesthetized normal dogs (15). Interestingly, the responses in normal dogs differ from those reported in man, in that higher doses of DDAVP are required to produce rises in plasma FVIII/vWF activities, and the observed increases, particularly of FVIII:C activity, are much less in dogs (10,11,12,13).

Desmopressin has been advocated as an alternative to blood component therapy in some forms of vWD and classical hemophilia in man (14). Our study on seven dogs with vWD has demonstrated substantial variability between dogs in the degree of response to DDAVP.

Desmopressin induced a rise in plasma FVIII:C activity in dogs with vWD comparable to that seen in normal dogs (26% and 38% increases in normal and vWD dogs respectively). The cause of the substantial elevations in plasma FVIII:C activity in man following DDAVP administration is not known although it has been postulated that it is the result of release of biologically active FVIII:C from storage and/or activation sites (17,18,19). Our studies with normal dogs suggest that, in comparison to man, dogs have a decreased ability to release biologically active FVIII:C when stimulated by DDAVP. Dogs with vWD, which have

significantly less plasma FVIII:C activity to begin with, are capable of responding as well as normal dogs when stimulated by DDAVP. Plasma FVIII:C activity usually ranges from moderately subnormal to normal (normal range 50-150%) in dogs with vWD (1,2). Desmopressin would likely be useful in normalizing FVIII:C levels in affected dogs with suppressed clotting levels.

Von Willebrand factor, a protein expressed antigenically as vWF:Ag and functionally as FVIII:RC is a product of vascular endothelium (5,6). The elevations in human plasma vWF:Ag and FVIII:RC activity induced by DDAVP are thought to be the result of release of preformed vWF from endothelial cells into the blood, rather than the consequence of increased vWF synthesis (20,21). Previous studies on normal unanesthetized dogs, and in people, supported the hypothesis of storage release since repeated short-term stimulation with DDAVP resulted in total or substantial reduction in the DDAVP-induced FVIII/vWF responses (15,21).

Five of the dogs with vWD used in this study had subnormal vWF as determined by both vWF:Ag and FVIII:RC measurements. Two dogs (dogs #3 and #4) had subnormal vWF:Ag but low normal or normal (54% and 70% respectively) levels of FVIII:RC activity. Desmopressin induced rises in vWF:Ag in all dogs, and in FVIII:RC in six of the seven dogs; the degrees of elevation however varied considerably.

Dogs with plasma vWF:Ag of <15% of normal demonstrated only minimal increases (20-60%) in this activity. The postinfusion plasma levels in these dogs remained below 30% of normal, a plasma concentration that has been considered necessary for relatively normal hemostasis (22). The dogs with pre-infusion vWF:Ag plasma levels of >15% of normal had much better responses to DDAVP with plasma vWF:Ag levels rising at least by 100%. This degree of increase was comparable to that in normal dogs. Our observations suggest that dogs with vWD who have very low plasma vWF:Ag levels (<15% of normal), likely have such limited storage reservoirs of this protein that there is little vWF available to be released by DDAVP.

Compared to plasma vWF:Ag activity, plasma FVIII:RC activity tended to increase more dramatically in the dogs with vWD, with increases ranging from 75-200% of the initial plasma concentration. One dog however (dog #7) showed no increase in FVIII:RC activity after DDAVP; the same dog showed the poorest vWF:Ag response. With this one exception, plasma FVIII:RC activity in the vWD dogs increased by an amount equal to or greater than the 80% increase observed in the normal dogs. It has been suggested that a vWF level in the plasma of at least 30% of normal is required for normal hemostasis (22). Our results indicate that DDAVP had a beneficial effect in six of the seven dogs as far as improving plasma FVIII:RC levels.

Neither plasma vWF:Ag or FVIII:RC activities increased significantly in dog #7 after DDAVP administration. All other vWD dogs however did show improvements in plasma vWF:Ag and/or FVIII:RC activity. In all of these responding dogs the rise in FVIII:RC (as a percent of initial plasma level) was substantially greater than the rise in plasma vWF:Ag. This observation suggests that there may have been an increase in the functional activity of vWF disproportional to the quantitative increase in vWF protein. This could reflect changes in the multimeric configuration of vWF as a result of DDAVP infusion. Ristocetin-induced aggregation (FVIII:RC activity) is primarily supported by high molecular weight multimers; multimers which are considered to be particularly important with respect to bleeding tendency (7,23,24,25,26). Low molecular weight multimers are less supportive of ristocetin-induced aggregation but are quantitated by immunoelectrophoresis. It is possible therefore that the disproportional elevation in FVIII:RC activity compared to vWF:Ag may be a reflection of the enhanced release of high molecular weight multimers by DDAVP.

Our study of DDAVP and its effects on dogs with vWD suggests that DDAVP may be useful in boosting the plasma level of FVIII/vWF activity in many dogs with this disease. Since the relationship between vWF:Ag and FVIII:RC; and actual bleeding tendency is not fully understood, it is uncertain as to how beneficial DDAVP would be in reducing the clinical bleeding tendency in dogs with vWD. Dogs #5, #6 and #7 each had a history of previous severe bleeding episodes either spontaneous or associated with surgery. All three underwent anesthesia and major surgery commencing immediately after the 60 minute sample. All surgeries were uneventful and no blood component therapy was required. There were rises in plasma vWF:Ag and FVIII:RC activity in dogs #5 and #6 (60% and 200%, and 44% and 75% increases respectively from pretreatment levels) that may have accounted for the 'apparent' improved hemostasis in these dogs. The FVIII:RC levels approached the 30% of normal level (21 and 28% respectively), however vWF:Ag remained significantly below the suggested minimal level (30%) for normal hemostasis. The 'apparent' improvement in dog #7 following DDAVP cannot be explained on the basis of increases in vWF quantity or quality as measured in this study. It may be that DDAVP improved hemostasis through mechanisms other than, or in addition to, correction of plasma FVIII/vWF deficiency.

The variation in the degree of increase in FVIII/vWF activity after administration of DDAVP to dogs with vWD, and the observation that repeat infusions into same recipient produced almost identical responses, suggests that pre-testing with DDAVP should probably be performed prior to using DDAVP as a prophylactic treatment in dogs with vWD requiring surgery.

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