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ORIGINAL ARTICLE

Effect of desmopressin on hemochromocytometric and clotting parameters in healthy blood donor dogs

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Summary
Six clinically healthy blood donor dogs were used to evaluate the effect of desmopressin (DDAVP) on haemochromocytometric and clotting parameters,. They received subcutaneously a physiological solution (placebo) and DDAVP (1μg/kg) with a two-weeks wash-out period between treatments. Blood samples were collected immediately before the injection, after 30 minutes, then after 1, 2, 3, 4, 5 and 6 hours. Two-way repeated measure analysis of variance (ANOVA) showed no significant effect of DDAVP vs placebo, except for white blood cell (WBC) count, and in particular on neutrophils. In conclusion, DDAVP administration in healthy donor dogs does not induce modification in blood cell count and clotting parameters, except of WBC.

Key words: desmopressin; clotting time parameters; hemochromocytometric parameters; dog; blood donor

INTRODUCTION

Blood-component therapy has become an integral part of veterinary practice. Blood donor dogs can save millions of lives each year. Each day dogs need blood for serious procedures from accidents, illness and surgery. In the last few years, veterinary blood banks have been established in many countries, and, although access to them has improved, some practitioners prefer to create their own blood-donor program to provide for their own blood-product needs or to respond to an emergency (Lucas et al. 2004). Choosing the appropriate dog as a donor is essential to the success of a transfusion (Hohenhaus 1992). Ideally, all donors have their von Willebrand factor (vWf) value within the reference range (Sato and Parry 1998). The drug of choice for the pre-treatment of human donors of blood in order to increase plasma factor VIII (FVIII)/vWf yields in the donor's plasma is desmopressin [DDAVP (1-deamino-8-D-arginine vasopressin)] (Lethagen 1997); this procedure is becoming of common practice in canine medicine (Sato and Parry 1998). DDAVP is a synthetic derivate of the naturally occurring antidiuretic hormone L-arginine vasopressin. In humans, its subcutaneous administration prior to plasma collection is a safe method of improving drastically the content of FVIII and vWf in cryoprecipitates (Randels et al. 1992, Palacios et al. 1995, Pomper et al. 2003). A number of studies have shown that DDAVP induces an elevation in plasma vWf concentration in normal

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dogs; however, the plasma FVIII activity does not appear to increase to the same level as in humans (Johnstone and Crane 1986, Kraus et al. 1989). In the dog, increases in the plasma content of FVIII and vWf seem to be dose-dependent (Johnson et al. 1986), and because of this characteristic, DDAVP is widely used for the treatment of patients with von Willebrand diseases, haemophilia A. It has also been used with good haemostatic effect in a variety of different disorders characterized by impaired platelet function and prolonged bleeding time, including congenital platelet dysfunctions, uraemia, liver cirrhosis and platelet dysfunction caused by drugs such as aspirin, both in human (Sutor 2000) and dog (Johnstone 1999, Sakai et al. 2003). The haemostatic effect of DDAVP probably involves additional cellular effects that remain to be discovered (Kaufmann and Vischer 2003). Köhler and Harris (2004) found that DDAVP has a pronounced effect on coagulation and fibrinolytic parameters in humans, and produces a rise in leukocyte counts. Tomasiak et al. (2004) found an increase in mean platelet volume and a decrease in platelet counts. In the dog, investigations have been performed only on coagulation parameters and PLT (Johnstone and Crane 1986, Sakai et al. 2003).

Studies of the DDAVP response in the dog have great potential in helping to define the mechanism and regulation of the stimulatory effect of DDAVP, not only in veterinary medicine but also in human medicine since the dog is commonly used as a laboratory animal model (Dodds 1981, Giles et al. 1982). The aim of this present study was to contribute to the knowledge of the effects of DDAVP on haemochromocytometric and clotting parameters in blood donor dogs.

MATERIALS AND METHODS

Six healthy blood donor dogs were used (Table 1). They were in good physical condition and without haemostatic disorders. All animals were fasted over night and then allowed to acclimatize to the testing area for at least 30 minutes prior to injection (09:30 am). Desmopressin (Minirin®/DDAVP injectable solution, Valeas, Milan, Italy) was administered subcutaneously as a single dose at 1 μg/kg of body weight. Two weeks before the start of the study they had received one subcutaneous injection of physiologic solution (placebo) at the same volume (0.25 ml/kg) of DDAVP, so that each animal served as its own control for the DDAVP administration. Blood samples were collected through cefalic venipuncture immediately before (basal) the DDAVP or placebo administration, after 30 minutes, 1, 2, 3, 4, 5, and 6 hours to assess haemochromocytometric and clotting profiles.

During the 12 hours after the DDAVP treatment all animals had no access to water to avoid hyponatraemia and the water intoxication due to the antidiuretic effect of this drug (Sutor 2000).

Blood samples were collected in K_3-EDTA tubes using polypropylene syringes and anticoagulated with K_3-EDTA or 3.8% sodium citrate. Immediately after the collection of these samples, a haemochromocytometric exam (Red Blood Cell – RBC, Hemoglobin – Hb, Haematocrit – PCV, White Blood Cell – WBC, Platelets – PLT) was carried out by means of an automated haematology analyzer (ADVIA 120 Hematology System, Bayer/Siemens, Germany). Blood samples collected in sodium citrate tubes, were centrifuged 15 min at 3000 × g, the plasma obtained was kept at +4 °C and clotting parameters (Prothrombin Time – PT, Actived Partial Thromboplastin Time – aPTT, fibrinogen concentration) were assessed within 2 hours of the sampling. The process was carried out with an automatic coagulometer (Clot 2, SEAC, Florence, Italy) according to the manufacturer's instructions and to standard protocol, to exclude differences that result from irregular test procedures.

Statistical analysis

Two-way repeated measures analysis of variance (ANOVA) was applied on the data obtained to evaluate the differences due to the administration of DDAVP or placebo and the significant effect of time on hemochromocytometric and clotting parameters. Where ANOVA showed an acceptable level of significance ($p<0.05$), Bonferroni's test was applied for *post hoc* comparison.

RESULTS

No dogs showed any sign of discomfort or pain during the study. They were amenable to repeated venipuncture and not visibly excited by the procedure. No side-effects, either local nor general, due to the DDAVP administration were observed.

Tables 2–3 show the time-course of the mean values and the SD of hemochromocytometric and clotting parameters in blood donor dogs.

The application of two way ANOVA showed no statistical significant modification due to placebo vs DDAVP administration on the haemochromocytometric and clotting parameters, except for WBC ($F_{(1,70)} = 11.01$, $p<0.01$) that showed

Table 1. **Six healthy donor dogs used as experimental study.**

Dog	Breed	Gender	Age (years)	Body weight (kg)
1	Labrador	Male	5	34
2	Crossbreed	Female	6	22
3	Crossbreed	Female	8	26
4	Crossbreed	Male	6	25
5	Pitbull	Female	7	30
6	German Shepherd	Male	5	35

an increase starting from 3 hours post injection (Fig. 1). This increase was within the reference range (Table 2), and involved the neutrophils ($F_{(1,70)}$ = 10.91, $p<0.01$). A statistically significant effect of time was observed on the following parameters: RBC ($F_{(7,70)}$ = 6.08, $p<0.0001$); Hb ($F_{(7,70)}$ = 6.14, $p<0.0001$); PCV ($F_{(7,70)}$ = 5.35, $p<0.0001$); WBC ($F_{(7,70)}$= 18.64, $p<0.0001$); neutrophils ($F_{(7,70)}$ = 26.97, $p<0.0001$); PLT ($F_{(7,70)}$ = 2.35, $p<0.05$); aPTT ($F_{(7,70)}$ = 2.63, $p<0.01$). Fibrinogen and PT showed no statistical significant modifications due to time: fibrinogen ($F_{(7,70)}$ = 1.79, p=0.11), PT ($F_{(7,70)}$ = 0.41, p=0.75).

DISCUSSION

It has been proposed that blood cells contribute to the haemostatic action of DDAVP (Sutor et al. 1980). Our results showed no effects of DDAVP administration on blood cell count levels, and other haemochromocytometric parameters. The only modification observed was a statistically significant increase of WBC, and in particular of neutrophils, starting at 3 hours after the DDAVP injection, and continuing until the end of the experimental period (6 hours). A 1.4–1.6 fold increase in WBC has also been observed in man 4 hours after subcutaneous or intravenous DDAVP administration (Köhler and Harris 1988), but the exact mechanism involved in this increase is not yet fully understood. In humans, contrasting results have been obtained concerning the ability of DDAVP to interact directly with PLT and to induce PLT modifications which may favour the haemostatic process (Balduini et al. 1999). It has been reported that DDAVP shortened prolonged bleeding time in human patients with aspirin-induced PLT dysfunction and prolonged bleeding, and activated a PTT in cases of chronic liver diseases (Cattaneo et al. 1990, Kam 1994, Manucci 1997). In dogs, the infusion of DDAVP at a dose of 0.6 μg/kg had no significant effect on PLT count and PCV, and a subcutaneous injection of DDAVP at a dose of 3 μg/kg in healthy dogs did not induce significant changes in PLT count (Sakai et al. 2003).

In blood donor dogs, DDAVP had no effect on clotting parameters. In fact, the change observed (a PTT decrease) both after placebo and DDAVP administration was probably due to an activation of the intrinsic pathway of coagulation induced by serial blood sampling.

Our results were in accordance with other studies carried out using DDAVP at a dosage of 0.2, 0.4 and 0.6 μg/kg after which increases in plasma concentrations of FVIII:C were not accompanied by significant changes in PT and aPTT (Johnstone and Crane 1986, Sakai et al. 2003).

It is proposed that DDAVP stimulates the release of a second messenger that results in the release of vWf from the vascular endothelial cell. It was also suggested that some are derived from WBC, such as platelet-activating factor (PAF), interleukin (IL)-6, or P-selectin (Hashemi et al. 1993, Mannucci 1997). In particular, PAF is a potent activator of both WBC and PLT (Kavakli et al. 2001), and P-selectin plays an important part in the "rolling" of polymorphonuclear leucocytes (PMN), as demonstrated in the mesenteric venules of rats (Kanwar et al. 1995).

In conclusion, we can claim that in healthy donor dogs subcutaneous administration of 1 μg/kg DDAVP is well tolerated and does not induce modifications of the blood cell count and clotting parameters, except of WBC. Further studies are necessary to better understand the effect of DDAVP on WBC, its duration and its involvement in haemostasis improvement.

Table 2. **Mean values ± SD of hemochromocytometric parameters after placebo or DDAVP administration in healthy donor dogs during all experimental conditions.**

Haemochrome	Placebo								
	Basal level	After 30 min	After 1 h	After 2 h	After 3 h	After 4 h	After 5 h	After 6 h	Reference range
RBC (M/µl)	7.72 ± 0.66	7.48 ± 0.68	7.54 ± 0.89	7.35 ± 0.92	7.24 ± 0.91	7.07 ± 0.75	7.34 ± 0.52	7.36 ± 0.84	4.5–6.5
HGB (g/dl)	18.55 ± 2.69	17.98 ± 2.38	18.10 ± 2.62	17.75 ± 2.91	17.62 ± 1.88	17.28 ± 2.44	17.50 ± 1.90	17.53 ± 1.45	13–18
PCV (%)	52.50 ± 7.38	50.65 ± 6.36	51.00 ± 6.90	49.65 ± 7.78	48.63 ± 5.68	48.03 ± 4.55	50.55 ± 5.74	49.65 ± 4.10	40–54
PLT (K/µl)	314.25 ± 104.55	312.75 ± 77.82	272.50 ± 101.80	312.75 ± 75.36	300.25 ± 80.74	302.25 ± 84.62	320.25 ± 78.70	306.50 ± 64.83	150–400
WBC (K/µl)	7.62 ± 0.94	7.48 ± 0.20	7.87 ± 0.20	8.13 ± 0.59	8.41 ± 0.87	8.48 ± 0.74	8.01 ± 0.58	8.12 ± 0.42	6–12
	DDAVP								
RBC (M/µl)	7.71 ± 0.61	7.23 ± 0.97	7.25 ± 0.94	7.19 ± 1.08	7.07 ± 0.80	6.80 ± 0.95	6.62 ± 0.90	6.74 ± 0.57	4.5–6.5
HGB (g/dl)	18.58 ± 1.98	17.58 ± 1.98	17.55 ± 1.98	17.32 ± 2.23	16.50 ± 0.89	16.25 ± 1.84	15.88 ± 1.62	16.18 ± 1.88	13–18
PCV (%)	51.80 ± 5.21	48.83 ± 5.36	48.80 ± 5.14	48.27 ± 6.32	47.50 ± 3.69	45.55 ± 4.60	44.38 ± 4.47	45.18 ± 5.04	40–54
PLT (K/µl)	326.75 ± 69.82	322.75 ± 85.25	305.00 ± 66.81	282.25 ± 46.72	317.50 ± 79.63	297.25 ± 70.86	289.50 ± 61.38	276.00 ± 69.24	150–400
WBC (K/µl)	7.40 ± 0.69	7.03 ± 0.62	7.28 ± 0.30	8.13 ± 0.42	9.41 ± 0.82	10.27 ± 0.60	10.45 ± 0.68	10.55 ± 0.32	6–12

Table 3. **Mean values ± SD of clotting profile after placebo or DDAVP administration in healthy donor dogs during all experimental conditions.**

Clotting profile	Placebo								
	Basal level	After 30 min	After 1 h	After 2 h	After 3 h	After 4 h	After 5 h	After 6 h	Reference range
PT (s)	7.58 ± 1.20	7.70 ± 0.80	6.95 ± 0.93	7.50 ± 0.84	6.83 ± 0.47	7.50 ± 0.78	7.32 ± 0.65	6.88 ± 0.86	5–8
aPTT (s)	13.48 ± 0.40	12.80 ± 0.86	11.78 ± 1.92	12.47 ± 1.89	12.80 ± 1.11	12.9 ± 0.76	13.65 ± 0.88	13.85 ± 0.61	9–20
Fibrinogen (mg/dl)	376.25 ± 90.85	288.28 ± 23.18	271.80 ± 17.34	243.65 ± 44.59	337.25 ± 124.33	288.00 ± 49.74	294.00 ± 45.69	282.38 ± 29.70	100–400
	DDAVP								
PT (s)	6.95 ± 1.20	7.73 ± 1.92	7.40 ± 0.98	7.28 ± 1.62	8.05 ± 1.70	6.82 ± 0.37	6.93 ± 0.37	7.08 ± 0.33	5–8
aPTT (s)	14.35 ± 0.85	13.45 ± 0.98	12.88 ± 0.17	12.40 ± 0.75	11.32 ± 2.32	13.50 ± 0.84	12.73 ± 0.30	11.62 ± 2.16	9–20
Fibrinogen (mg/dl)	304.10 ± 14.32	300.03 ± 12.28	274.23 ± 19.86	284.50 ± 28.98	310.90 ± 49.69	324.07 ± 94.26	337.43 ± 77.54	281.05 ± 18.35	100–400

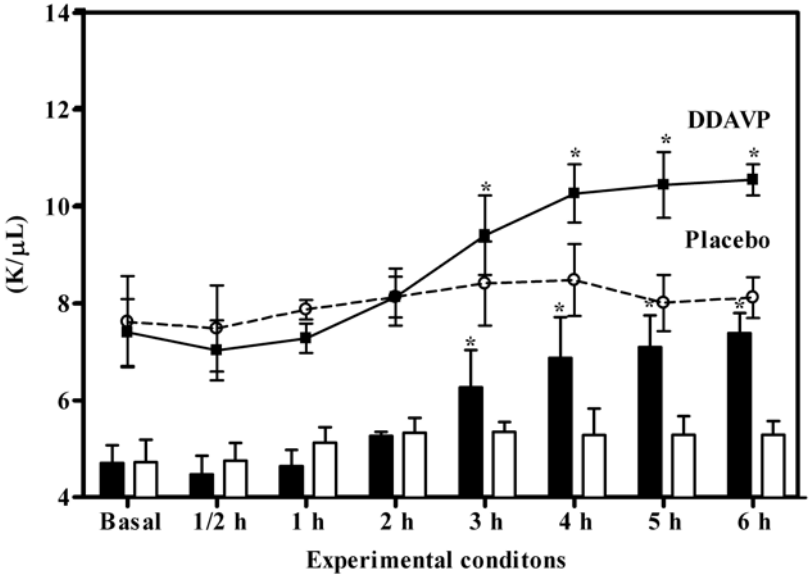

Fig. 1. **Mean pattern ± (SD) of WBC (lines) and neutrophils (columns) before and after DDAVP (■) or placebo (□) administration.** Significance: *vs basal $p<0.001$**.**

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