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Platelet function in dogs with congenital portosystemic shunt

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ABSTRACT

The aim of this study was to examine the influence of congenital portosystemic shunts (CPSS) on primary haemostasis in dogs. Bleeding time, automated platelet function analysis (PFA 100 analyser), platelet count and platelet aggregation using different methods and agonists were measured in 10 dogs with untreated CPSS and in 10 healthy, age-matched controls.

Bleeding time, platelet function analysis and platelet counts did not differ significantly between groups ($P > 0.05$). Aggregation measured using the impedance method (area under the curve) was slightly to moderately reduced with high concentrations of collagen (e.g., 5 µg/mL: 2948 ± 524 vs. 3472 ± 571 AU ∗ Min) and arachidonic acid (e.g., 1 mmol/L: 1006 ± 522 vs. 1963 ± 738 AU ∗ Min) ($P < 0.05$), but not with adenosine diphosphate. In contrast, collagen-induced turbidimetric aggregation revealed slightly higher maximum aggregation values in dogs with CPSS. Despite the moderately altered platelet aggregation, the lack of change in global primary haemostasis screening tests indicates that dogs with CPSS do not have regularly occurring clinically relevant disorders of primary haemostasis.

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Introduction

Congenital portosystemic shunt (CPSS) is a common vascular malformation in dogs caused by a persistent fetal vessel that connects the portal vein to the systemic venous system circumventing the liver (White and Burton, 2000). This vascular anomaly causes decreased liver function resulting in a number of clinical signs, which predominantly affect the central nervous system, the gastrointestinal tract and to a smaller extent the urinary tract (Mehl et al., 2005). In addition, it is associated with increased peri-operative bleeding tendency (Willis et al., 1989; Kummeling et al., 2006).

Different studies on large numbers of dogs with CPSS have investigated the plasmatic coagulation system and platelet counts and have shown decreased coagulation factor activity, prolonged clotting times and decreased platelet counts (Kummeling et al., 2006; Niles et al., 2001). In contrast, the possible contribution of altered platelet function to increased bleeding tendency is not as well documented and with partly conflicting results (Willis et al., 1989; Schulze, 1998; Keidel, 2001). The aim of this study was to assess primary haemostasis in dogs with untreated CPSS using different global tests and specific methods in comparison to an age-matched control group.

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Materials and methods

Study design

Ten dogs with untreated CPSS and an age-matched control group were studied and compared regarding the following parameters of primary haemostasis: capillary bleeding time, automated platelet function analysis using a PFA 100 analyser, platelet count, turbidometric and impedance platelet aggregometry using different agonists, and haematocrit. The experimental design was approved by the ethics committee of the responsible agency (Lower Saxony State Office for Consumer Protection and Food Safety; reference number 07A 514).

Animals

All 10 dogs with CPSS were patients of the Small Animal Clinic, Hannover School of Veterinary Medicine. Diagnosis was based on clinical symptoms, increased plasma ammonium concentrations, detection of the shunt vessel with abdominal sonography and/or computer tomography-angiography (nine cases) and/or as a surgical finding including histological examination (eight cases). Plasma ammonium concentration after a 12 h fast was significantly elevated in all dogs (median 338 µg/dL; minimum–maximum 199–496 µg/dL). Eight dogs had intra-hepatic (5 right-divisional, 3 left-divisional) and two dogs extrahepatic, portocaval shunts. Dogs with CPSS were of mean age 5 months (range 3–9 months). To minimise the influence of possible secondary organ dysfunctions, the only exclusion criterion from the study was if the dog's age was >1 year. Apart from one mongrel, there were two Golden Retrievers and one dog from each of the following breeds: Beagle, Boxer, Czechoslovakian Wolfdog, English Bulldog, French Bulldog, Great Dane and Pug. Eight dogs were male and intact, one was an intact female, and there was one neutered female.

The control group consisted of 10 privately owned, clinically healthy dogs. This group included 3 mongrels and 7 purebred dogs (2 Belgian Malinois, 2 German Shepherds, 1 Hanoveranian Scenthound, 1 Labrador retriever, and 1 Staffordshire terrier) of different sexes (3 intact males and 7 intact females). The group was primarily selected to achieve a similar age distribution (6 months, 2–10 months) to that of the CPSS-group ($P = 0.545$).

Blood sample collection

Blood samples were obtained from the saphenous, cephalic or jugular vein and only slight pressure was used to raise the vein. Blood was collected (1) into plastic tubes containing buffered citrate solution (3.8 mL S-monovette tube 0.129 mol/L trisodium citrate/citric acid buffer solution pH 5.5, Sarstedt) for measurements with the platelet function analyser, (2) into plastic tubes covered with hirudin to measure platelet aggregation with the impedance method (namely a 4.5 mL thrombin inhibitor blood collection tube suited for the S-Monovette system, Dynabyte Medical), (3) into plastic tubes containing 0.11 mol/L citrate solution (one part for nine parts of blood) for the preparation of platelet rich plasma (PRP) to perform the turbidimetric aggregometry, and (4) into EDTA-coated tubes for platelet count and haematocrit measurements. Blood and anticoagulant were immediately and thoroughly mixed by careful swaying. Measurements were performed within 3 h following collection of blood samples. During that time, blood samples were kept at room temperature.

PRP for the turbidimetric aggregometry was produced by centrifuging whole blood samples at 250 *g* for 15 min. Part of the plasma was transferred to 1.5 mL plastic tubes and centrifuged again for 10 min at 16,000 *g* to achieve platelet free plasma (PFP).

Capillary bleeding time

The capillary bleeding time was measured according to the method described by Nolte et al. (1997) in non-anaesthetised dogs in lateral recumbency using the lateral side of a front toe close to the edge of the horny skin of the pad with a sterile semi-automatic blood lancet (Softclix II, Roche Diagnostics). While steady pressure was maintained, the blood was carefully dabbed off every 10 s with a swab until the bleeding stopped. The average bleeding time was calculated from the two parallel punctures.

Platelet function analyser (PFA 100)

The platelet function analyser PFA-100 (Siemens Healthcare Diagnostics) aspirates the blood sample under constant vacuum from the sample reservoir through the capillary and a microscopic aperture in the membrane located at the end of the capillary which is coated with collagen and either epinephrine bitartrate (collagen/epinephrine cartridge) or adenosine 5′-diphosphate (collagen/ADP cartridge). The instrument provides a quantitative measurement of platelet function in anticoagulated blood (Kundu, 1995). Measurements were performed as recommended by the manufacturer. The instrument automatically reported the time required to obtain full occlusion of the aperture as 'closure time' (in s) and the required blood volume until this time as 'total volume' (in µL). The upper limit of the measurement range was 300 s.

Platelet count and haematocrit

Haematocrit, blood and PRP platelet counts were measured automatically using a blood cell counter (ADVIA120, Siemens Healthcare Diagnostics). The instrument detects platelets and red blood cells using a double angle laser light scattering procedure after isovolumetric sphering.

Impedance aggregometry

Whole blood platelet aggregation was measured using an impedance aggregometer, the Multiplate analyser (Dynabyte Medical; Calatzis, 2007) which was recently evaluated for canine samples (Kalbantner et al., 2010). The test procedure was performed as recommended by the manufacturer (i.e., 300 µL isotonic sodium chloride, 300 µL hirudin-anticoagulated blood [1:2 dilution]; 3 min of incubation; 20 µL agonist solution), except that a test time of 12 min was used instead of 6 min. All agonist reagents were sourced from Dynabyte Medical and used in the following final concentrations: 1, 5, 7.5, 10 and 20 µmol/L ADP; 0.5, 3, 5 and 10 µg/mL collagen, or 0.5 and 1 mmol/L arachidonic acid. Of the different parameters provided by the machine, the 'area under the curve' (AUC) value (in aggregation units [AU] * min), which expresses the aggregation response over the registration interval, was reported.

Turbidimetric platelet aggregation

The turbidimetric technique described by Born (1962) is based on the recording of the changes in optical density (increased light transmission) after adding agonists to the PRP and was carried out using a four-channel aggregometer (APACT 4004, Rolf Greiner Biochemica) with computer-based curve analysis.

The sample specific calibration was performed with two standards. The first standard (100% aggregation, corresponding to the light transmission of PFP) was achieved by adding 20 µL isotonic NaCl to 200 µL PFP. The second standard (0% aggregation) was prepared by adding 20 µL isotonic NaCl to 200 µL PRP. Measurement of samples was performed by incubation of 200 µL PRP for 2 min at 37 °C before induction of aggregation by adding 20 µL platelet agonist containing solutions (Dynabyte) to achieve final concentrations of 10, 20 or 40 µmol/L ADP or 10, 20, 40 µg/mL collagen. The parameter maximum aggregation (%) was calculated automatically from the aggregation curve, which was recorded for 12 min.

Statistical analysis

Data were tested for normal distribution using Kolmogorov–Smirnov test. Due to the fact that all the examined parameters showed normal distribution, results are presented as mean ± SD (minimum and maximum values are given additionally) and comparison of the two groups was performed by using a *t* test for independent samples. $P < 0.05$ was considered significant. Statistical analysis was performed using SPSS 16.0 German (SPSS Inc.).

Results

There was no significant difference between dogs with CPSS and normal dogs with respect to capillary bleeding time, platelet function analysis, platelet count, and ADP-induced platelet aggregation and extreme values exceeded the range of the age-matched controls only minimally (Table 1). AUC values of impedance aggregometry using 3, 5 or 10 µg/mL collagen as agonist were significantly lower in dogs with CPSS when compared to controls. More pronounced differences were seen with 1 mmol/L arachidonic acid where the AUC values in CPSS dogs were only approximately half as high as those in control animals. Interestingly, aggregation maximum values detected by the Born method using 40 µg/mL collagen were significantly higher in dogs with CPSS than in controls.

Discussion

The present study did not reveal significant changes in capillary bleeding time or in results using the platelet function analyser PFA 100 in dogs with CPSS. Both methods are approved screening methods for primary haemostasis function in dogs (Nolte et al., 1997; Callan and Giger, 2001; Mischke and Keidel, 2003). Therefore, our results indicate that clinically relevant alterations in primary haemostasis do not occur regularly in dogs with CPSS and are unlikely to be a major cause of increased peri-operative bleeding seen in this disease. This is especially significant, because dogs with CPSS had significantly lower haematocrit values, which cause prolongation of the closure time of the platelet function analyser (Callan and Giger, 2001; Mischke and Keidel, 2003).

Our results partly confirm those reported by Keidel (2001). In that study also using 10 dogs with CPSS, median closure times of the collagen/ADP cartridge were identical between CPSS and control dogs (72 vs. 73 s; $P < 0.379$), but increased median closure times were observed in CPSS dogs with the collagen/epinephrine cartridge (212 vs. 163 s; $P < 0.021$). Keidel (2001) and other studies (Willis et al., 1989) used reference values established for adult dogs, whereas CPSS dogs usually present at a young age. Age influences the results of platelet aggregation tests in humans and laboratory animals (Gleerup and Winther, 1988; Okazaki et al. 1998), but studies on humans did not show significant differences for the automated platelet function analysis between children and adults (Carcao et al., 1998).

Regardless of the results of the screening tests, moderate alterations were seen in platelet aggregation tests in CPSS dogs using collagen and arachidonic acid as agonists, but not with ADP. Willis et al. (1989) also found a selective reduction of collagen- and arachidonic acid-induced whole blood platelet aggregation. These authors studied whole blood platelet aggregation with the agonists collagen, ADP and arachidonic acid in a total number of 20 dogs with hepatic diseases, including 11 dogs with CPSS, but did not report the results for the CPSS dogs and used adult dogs as controls.

Possible causes of platelet function disorders related to CPSS include inhibiting effects of hyperammonaemia and changes in

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