Assessment of coagulation function by thromboelastography in dogs with mitral valve insufficiency

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In veterinary medicine, a variety of disease are known to cause coagulation abnormalities. Identification of these coagulation abnormalities have been relied on traditional coagulation assays(platelet concentration, aPTT, PT, D-dimer, fibrinogen) which take only a small part of the coagulation pathways rather than global hemostatic capacity. Among of the hypercoagulable diseases, cardiovascular disease, such as mitral valvular disease, was not regarded as the cause of the hypercoagulability. The value of a thromboelastography (TEG) as an early predictor of coagulopathy, especially hypercoagulability, has been founded. It was associated with decreased R and K values, and increased MA and α angle. The objective of this study was to compare thromboelastography results and those of traditional coagulation tests between twenty adult dogs with mitral insufficiency (MVI group) and eleven adult healthy dogs (Healthy group). As a results, MA values in the patients with mitral insufficiency (68.8±7.8 mm) were significantly higher than the normal patients (60.4±4.8 mm) (*P* value<0.05). Although a little report has been reported in veterinary medicine, platelet activation seems to be related with hypercoagulability in MVI patients in human medicine. The result of this report can support this pathophysiology in veterinary medicine. In addition to traditional coagulation assay, global assessment of coagulopathy using TEG, especially ability to detect hypercoagulability, may be useful for customized treatment in MVI patients. To achieve this, further study is needed to define pathophysiology and effect of medication.

Key Words: Coagulation function, Dog, Mitral valve insufficiency, Hypercoagulability, Thromboelastography

INTRODUCTION

The most common cause of canine heart failure is chronic degenerative atrioventricular valve disease which is estimated to be over 70% of the cardiovascular disease recognized in this species. The mitral apparatus is affected most often, however, isolated myxomatous degenerative disease of tricuspid valve, aortic and pulmonic valve is less frequently involved in veterinary medicine.

Valvular heart disease can affect platelet activation or function in consequence of turbulent high-velocity blood flow and fluid shear stress. Initially, increase of activation and reactivity of platelet would be expected.

However, lasting stimulation and stress may lead to structural and biochemical changes which related to decreased platelet function (Brown et al, 1975). In human studies, alteration of platelet function with heart disease lead to the progression of vascular remodeling, thromboembolism and fatalities (Michelson 2004).

The relationship between thromboembolism and mitral valve disease (MVD) in veterinary medicine is a lesser extent, but the recent study added up knowledge regarding the hemostatic markers in dogs with MVD. Increased fibrinogen and D-dimer and decreased AT III and protein C activities are the most important factors (Prihirunkit et al, 2014). Identification of these abnormal coagulation status has been relied on traditional

Chorok Jeong · Minwoong Seo · Ocki Chang · Jinho Park · Chul Park

plasma-based coagulation assays including aPTT, PT, AT activity, FDP, D-dimer and fibrinogen which fail to take overall hemostatic capacity into consideration and possess only a small part of the coagulation pathways (Wiinberg et al, 2008; Wagg et al, 2009). And identifying hypercoagulable state with traditional coagulation tests is difficult, time and cost-consuming due to variable factors of hypercoagulability disease and it needs expertise to interpret a dog's comprehensive coagulation status as well as to predict the risk of thromboembolic disorders (Wagg et al, 2009). If coagulation abnormalities are often encountered in dogs with concurrent diseases related with hypercoagulability and if not prevented early may have a fatal of thromboembolic complications which are much more difficult to treatment and closely related with severe mortality (Crowell and Read, 1955; New and Byers, 2011).

Although thromboelastography (TEG) is not new method, its potential use in assessing hemostatic disorders has resurfaced after the assay was automated and new activators were introduced, allowing for rapid and global assessment of hemostatic function in dogs (Donahue and Otto, 2005; Wiinberg et al, 2008; Trapani 2013). TEG provides a comprehensive contours of the clotting process, from initial thrombin creation to formation of fibrin to fibrinolysis (Donahue and Otto, 2005). The graph of TEG shows initial fibrin creation to fibrinolysis using small amount of whole blood and bring veterinarian up to the information of the whole hemostasis process of patients (Bowbrick et al, 2000). Consequently, TEG can monitor antithrombotic therapies, and help prevent risks of hyperthrombosis and bleeding (Nielsen et al. 2004). The values of TEG are reaction time (R), clot formation time (K), alpha angle (α) and maximal amplitude (MA) (Fig. 1). As for terminology of TEG, R is the time in minutes from clot initiation until the first fibrin polymers are produced and the amplitude reaches 2 mm. And K is the time in minutes from the end of R until an amplitude of 20 mm is reached and represents the speed of clot formation. Next, MA is the maximum amplitude in millimeter and reflects
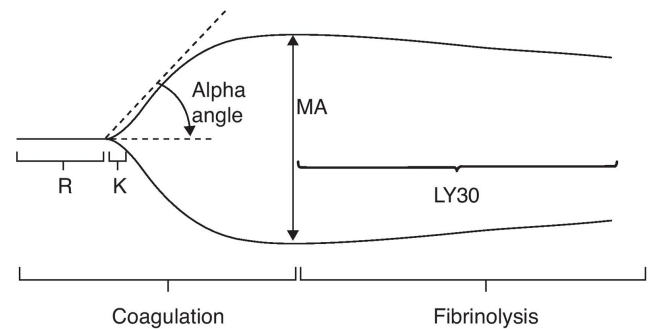
Fig. 1. TEG graph. X-axis represents time in minutes and Y-axis is amplitude of pin rotation in millimeters. R is the time that it takes for the tracing to reach the amplitudes of 2 mm, and K is the time in minutes from the end of R until and amplitude of 20 mm. Alpha is angle in degree tangent to the curve as K is reached. MA is the maximal amplitude of the tracing that reflects maximal strength of the clot.

maximal clot strength. Lastly, α is the angle in degree tangent to the curve as K is reached and represents the acceleration of fibrin formation and crosslinking (Kol and Borjesson, 2010). The value of TEG as an early predictor of coagulation state, especially hypercoagulability, has been published. Hypercoagulability should be associated with decreased R and K values, increased MA and α angle (Donahue and Otto, 2005).

The objective of this study was to compare TEG results and those of traditional coagulation tests between dogs with cardiovascular system disease such as mitral valve insufficiency and healthy control dogs.

MATERIALS AND METHODS

Animals and blood samples collection

Twenty adult dogs with mitral insufficiency (Patient group; 9 male dogs and 11 female dogs; mean age 11.3 years, range 5~16 years) and eleven adult healthy dogs (Control group; 6 male dogs and 5 female dogs; mean age 9.3 years, range 6~14 years) were involved in this study. Control group dogs were approved to be healthy based on absence of clinical signs, physical examination, general blood test. Coagulation test was done in both groups. Coagulation profile included platelet

counts, D-dimer, PT, aPTT and fibrinogen concentration. Using a 23-gauge needle with 5 mL syringe, 5 mL of blood was collected from jugular vein and transferred into EDTA tube and 3.2% sodium citrate tube. Platelet concentration measurement using CBC test were performed within 1 hour of sampling with EDTA whole blood. Citrate whole blood were used to analyze traditional coagulation test and TEG analysis. Residual plasma sample of citrate tube were frozen at −20℃ and sent to veterinary diagnostic laboratory (Neodin vetlab, Seoul, Korea) to measure fibrinogen concentration within 1 week.

Thromboelastographic analysis

The TEG analysis was performed using citrated whole blood samples with a computerized thromboelastography (TEG® 5000 Thrombelastograph®, Haemoscope Corporation, Niles, IL, USA). For TEG analysis, thromboelastography pin and cup supplied by the manufacturer was loaded in 1 channel, and 20 μL of calcium chloride was added to the cup. And then, 1 mL of citrated whole blood from all dogs were transferred to kaolin-coated vial, and 340 μL of this samples were mixed with calcium chloride. Next, the cup was gently raised into its respective pin, and TEG measurements was initiated. The calculated parameters from the resulting trace were 1) R time, 2) K time (clot formation time) which is the time in minutes to reach a 20 mm in amplitude, 3) α angle that represents the rate of fibrin build-up and cross-linking, 4) MA (maximum amplitude) which correlates to platelet counts and activity and 5) the time in seconds from initiation to the measurement of MA.

Traditional coagulation tests

Platelet concentration, PT, aPTT, plasma D-dimer and fibrinogen were assessed. Platelet concentrations were evaluated with an automated complete blood count instrument (MEK-6450, Nihon Kohden, Tokyo, Japan), PT and aPTT with an automated blood coagulation analyzer (IDEXX Coag Dx analyzer, IDEXX Laboratories, Westbrook, Maine, USA), and concentration of D-dimer with immunometric assay (AXIS-SHEILD PoC AS, Oslo, Norway) using the gold-antibody conjugate principle.

Statistical analysis

The statistical analysis was performed using statistical software (IBM SPSS statistics 22.0, SPSS Inc, USA). An independent t-test was used to compare the TEG parameters between dogs with mitral valve insufficiency and healthy, control dogs. A P-value<0.05 was considered statistically significant.

RESULTS

Traditional coagulation test (platelet, aPTT, PT, D-dimer, fibrinogen) was performed concurrently with TEG analyses in 31 dogs of healthy group (n=11) and MVI group (n=20) in this study. The mean±SD of traditional coagulation test and TEG value were described in Table 1, 2. Traditional coagulation panel values of healthy / MVI group were : Platelet=254.8±50.9 / 409.3±155.4, aPTT=23.3±3.5 / 26.4±4.4, PT=10.2±0 / 10.18±0.04, D-dimer=0.35±0.04 / 0.73±0.58 and Fibrinogen=203.2±32.8 / 332.1±109.8 (Table 1). All of the traditional coagulation panel values were within the reference range except for fibrinogen value of patient group. And there

Table 1. Results of traditional coagulation panel on healthy group (n=11) and MVI group (n=20)

Variable	Ref. range	Group	Mean±SD
Platelet (10^9/L)	200～500	Healthy group	254.8±50.9
		MVI group	409.3±155.4
aPTT (sec)	20.0～42.0	Healthy group	23.3±3.5
		MVI group	26.4±4.4
PT (sec)	6.0～11.0	Healthy group	10.2±0
		MVI group	10.18±0.04
D-dimer (mg/L)	0.0～0.25	Healthy group	0.35±0.04
		MVI group	0.73±0.58
Fibrinogen (g/L)	109～311	Healthy group	203.2±32.8
		MVI group	332.1±109.8

Table 2. Results of TEG value on healthy dogs (n=11) and MVI group (n=20)

Variable	Ref. range	Group	Mean±SD	P-value
R(min)	2~4	Healthy group	3.8±1.3	0.603
		MVI group	4.1±1.6	
K(min)	1~2	Healthy group	1.8±0.6	0.132
		MVI group	1.4±0.6	
Angle (deg)	60~74	Healthy group	65.9±6.6	0.096
		MVI group	70.4±7.1	
MA(mm)	50~62	Healthy group	60.4±4.8	0.003[†]
		MVI group	68.8±7.8	

Angle, Kinetics of clot development; K, clot formation time; MA, maximum amplitude; R, reaction time. ([†]Statistically significant level was set as $P<0.05$).

are no significant differences in all type of panel. TEG values of healthy / MVI group were: R=3.8±1.3 / 4.1± 1.6 (min), K=1.8±0.6 / 1.4±0.6 (min), Angle=65.9±6.6 / 70.4±7.1 (degree) and MA=60.4±4.8 / 68.8±7.8 (mm) ($P=0.003$, Table 2). Values of R and MA of MVI group were outside the reference range and there is significant difference between MA values of healthy and MVI group (Table 2).

DISCUSSION

In human medicine, coagulation evaluations by TEG are used to evaluate hemostatic conditions such as post-operative hemorrhage following cardiovascular surgery or thrombosis during organ transplantation surgery (Holcomb et al, 2012). And these reports indicated that hypercoagulability is the state when the hemostatic balance is changed towards procoagulant inducing excessive platelet activation and fibrin deposition, which lead to thrombosis. In veterinary medicine, hypercoagulable states evaluated by thromboelastography have been reported suffering from protein-losing nephropathies (PLN) and enteropathies (PLE), hyperadrenocorticism, neoplasia (Kristensen et al, 2008; Goodwin et al, 2011; Kol et al, 2013; Lennon et al, 2013). Goodwill et al. reported that dogs with PLN have hypercoagulable state of TEG values compared with a control group, MA was significantly higher in PLN than in control dogs

(Lennon et al, 2013). While the predisposition to thromboembolism in dogs with protein-losing nephropathy, the underlying cause of hypercoagulability is incompletely understood. The deficiency of antithrombin and increased aggregation of blood platelets secondary to hypoalbuminemia have been demonstrated (Green and Kabel, 1982; Green et al, 1985). PLE allows leakage of protein into the intestinal lumen and thromboembolic disease can develop in consequence of PLE (Peterson and Willard, 2003). Similar to the PLN, enteric loss of antithrombin (AT) might induce hypercoagulability, but there is little evidence to support this suggestion (Vaden et al, 2000). In one TEG study of PLE suggested that prevalence of hypercoagulability in dogs with PLE is higher than healthy dogs (Goodwin et al, 2011). Next, the result of previous study about TEG trends in dogs with naturally occurring hyperadrenocorticism showed very similar result to our experiment (Kol et al, 2013). The majority of the dogs in Kol et al. study continued to have increased MA even though their fibrinogen concentration was normalized. Kol et al. suggested that fibrinogen concentration is not solely responsible for increased MA noted in canine hyperadrenocorticism patients.

Thrombus in mitral valve insufficiency was formed and adhered at the cul-de-sac which created between the ballooning posterior leaflet and the atrial wall (Falicov and Resnekov, 1977). Possible mechanism of platelet activation associated with mitral valve disease may be due to hemodynamic irregularities (turbulent flow in the left atrium) caused by the regurgitation jet in the presence of an abnormal valvular surface, independent of the underlying etiology. The activated platelet may then adhere and aggregate on the abnormal mitral valvular surface to form a platelet-fibrin thrombus on the leaflet, potentially resulting in thromboembolic events (Tse et al. 1997).

The traditional coagulation panel, plasma D-dimer is a fibrin degradation product and a marker of increased fibrin production or increased fibrinolysis and increase in the plasma concentration of D-dimer has been re-

ported with cardiovascular disease and thromboembolic disease, compared with concentrations in unaffected individuals. The aPTT and PT are measures of the overall coagulation process. But unfortunately, assessment of hypercoagulability and thrombosis in dogs is very difficult with routinely used traditional coagulation assays such as D-dimer, aPTT and PT. Endogenous antithrombotic ability has been evaluated through measurement of antithrombin activity and concentrations of protein C and protein S (Feldman et al, 2000). Specialized individual coagulation factor tests can be performed to further localize the defect. All of these tests of the secondary and fibrinolytic systems are performed on citrated plasma samples and target very specific elements in the hemostatic system and thus potentially discount important cellular factors. Although this approach makes it possible to diagnose the status of coagulation abnormalities effectively and systematically, however, it can be difficult from a clinical perspective to piece together a picture of a dog's overall hemostatic capability and to predict or monitor the effect of treatment with anticoagulant or procoagulant medication with this traditional approach, especially if the dog is suspected of being hypercoagulable (Wiinberg et al, 2008). TEG has also been cited in a few abstracts, but the total amount of published material on dogs is sparse. TEG is increasingly used to monitor hemostatic function in humans after cardiac surgery and to optimize blood-product selection and usage, but the role of TEG also includes platelet-mapping assays as well as diagnosis and treatment of both hypo- and hypercoagulable states.

In the present study, the results revealed that dogs with mitral valve insufficiency represent a trend toward hypercoagulability with greater angle α and increased MA than healthy control dogs. This trend indicates that dogs with mitral valve insufficiency have increased clotting activity and have a tendency of higher clot strengths. Several previous studies had been reported that an increased MA which reflects final clot strength is strongly related with platelet activation and fibrin binding (Wiinberg et al, 2008; Wagg et al, 2009; Lennon et

al, 2013). Intriguingly, most dogs of patient group with increased MA value which indicative of hypercoagulable status had a platelet count that was within a reference range. As result of present study, certain correlation between MA value and platelet count in dogs with underlying disorders associated with a hypercoagulable status could not find. These results suggest that MA value is not influenced by the platelet count, but by the ability of platelet bonding. And greater angle α is presumed to be related to be increased of fibrinogen in traditional coagulation panel. These results represented that the dogs with mitral valve insufficiency had a much complicated hemostatic factors and that TEG may be advanced diagnostic tool in assessing the overall hemostatic status and feasible to predict or monitor the effect of treatment with anticoagulant or procoagulant medication, especially if the dog is suspected of being hypercoagulable such as cardiovascular disease.

The limitation of this study was the small number of dogs in this study. R value in present study was not significantly different between the MVI group and the control group which was considered the small number of dogs in the experimental groups, which might have affected the significance of the differences. Further prospective studies involving larger numbers of dogs with hypercoagulable diseases are necessary for tracing the pathogenesis to thromboembolism with clinical signs of hypercoagulable or procoagulable status through the results of their TEG analysis. Moreover, if a much number of experiments are performed, the value of the α angle can be changed significantly in the further experiment. And there are many methods that can be used to evaluate platelet function were reported (Olsen et al, 2001; Bowbrick et al, 2003; Tong et al, 2016). We need for comparison between TEG value and other tests for some more precise assessment of platelet function.

In conclusion, with the ability to detect hypercoagulability, TEG provides the clinician with the unique ability to identify dogs that are in the proinflammatory and hypercoagulable state and offer the novel possibility of clearly differentiating affected dogs from cardiovascular

disease, and as such, TEG may potentially be useful for individualization of treatment. This study suggested that fibrinogen concentration, ability of platelet bonding and angle α value, MA are correlated, respectively, but increased fibrinogen concentration is not solely responsible for the increased MA. Nevertheless, this finding is supported by results of studies in humans, demonstrating that platelet activation is of critical importance in the pathophysiology of MVI induced hypercoagulability. Further studies are needed to address whether hypercoagulable dogs with another cardiovascular disease will benefit from therapeutic intervention by anticoagulant therapy, and whether TEG can be used to guide and individualize such treatment.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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