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A prospective evaluation of oral Yunnan Baiyao therapy on thromboleastographic parameters in apparently healthy dogs

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Abstract

Objective – To determine the effect of Yunnan Baiyao (YB) on hemostatic parameters measured by thromboelastography (TEG) in healthy dogs administered 1 capsule of YB orally twice daily for 1 week.
Design – Prospective study of client-owned dogs at a small animal specialty hospital.
Setting – Private referral center.
Animals – Eighteen client-owned adult dogs weighing at least 15 kg.
Interventions – Dogs had a baseline TEG performed and then each dog was administered 1 capsule of YB twice daily by mouth for 1 week and the TEG was reevaluated. Any side effects attributed to YB were noted at this time.
Measurements and Main Results – All 18 enrolled dogs completed the study. Dogs that received 1 capsule (250 mg/capsule) of YB orally twice daily had significantly increased G as well as A30 and A60 values. There was also a significantly decreased LY30 and LY60 values after 1 week. The YB appeared well tolerated as only one dog developed mild diarrhea.
Conclusions – The results of this study suggest that YB at 1 capsule orally twice daily in healthy medium to large breed dogs increases the strength of the clot as measured by TEG and that YB was apparently well tolerated in the study population reported here. Larger prospective studies in different disease states are warranted to further evaluate these preliminary findings.

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Keywords: hemostatic agent, procoagulant, viscoelastic testing

Abbreviations

TEG	thromboelastography
YB	Yunnan Baiyao

Introduction

Yunnan Baiyao (YB) is a traditional Chinese medicine herbal remedy used to reduce bleeding by topical or oral administration. Following administration in people and noncanine species, significant decreases in bleeding and clotting times have been observed.[1–5] In human studies, significant decreases in bleeding were observed in patients with different types of cancers and ulcerative diseases when YB was used in conjunction with conventional hemostatic interventions.[6,7] In noncanine species, YB produces a hemostatic improvement via enhanced expression of surface glycoproteins on platelets under conditions of stimulation, shortening bleeding times.[1,2,7,8] Surface glycoproteins found on the platelet cell membranes have a diverse range of functions that involve hemostasis, inflammation, antimicrobial host defenses, and angiogenesis. GPIIb/IIa or integrin $\alpha_{IIb}\beta_3$ is one of the most important glycoproteins involved in hemostasis mediating platelet aggregation via fibrinogen binding and is one of the major mechanisms controlling platelet adhesion to the extracellular matrix.[9–11] It is hypothesized that YB could prove useful in clinical conditions of uncontrolled bleeding such as that following

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from trauma or vascular tumors (eg, hemangiosarcoma), although it is unclear if such hemostatic benefits occur in dogs administered YB.

Conventional coagulation tests usually evaluated limited contributors to hemostasis, generally reflecting tendency toward hypocoagulability. Thromboelastography (TEG), however, is a viscoelastic test that evaluates multiple components contributing to coagulation and is considered a global test of hemostasis. TEG measures several variables including the R time, K, alpha, and MA. The R time corresponds to the initiation phase and reflects activity of factors VIII, IX, XI, and XII.[12,13] K reflects amplification phase and demonstrates clot formation time, it is influenced by factor II, VIII, platelet numbers, thrombin formation, fibrin, fibrinogen concentrations, and hematocrit. The alpha angle is the point at which the amplitude of the trace reaches 20 mm and is affected by similar factors as K. The MA is indicative of the final clot strength and is influenced by fibrin, fibrinogen concentrations, platelet numbers and function, thrombin, factor XIII activity, and hematocrit. The amplitude (A) measures the width of the TEG trace at its latest time point and is a function of clot strength and elasticity at 30 (A30) and 60 minutes (A60). The G-value is a log derivation of the MA and is meant to also reflect clot strength. These values can be entered into a formula to derive the coagulation index where values above 3.0 are considered hypercoagulable and those below −3.0 are hypocoagulable, although this index has never been validated in animals.[12,13] Consequently, a TEG may be a useful means to determine if YB produces any measureable improvement in hemostatic function in dogs. A previous small study found a significant increase in only R values when pre- and posttreatment TEG parameters were compared in 8 Beagles receiving YB.[a] The primary objective of this study was to evaluate if administration of YB was associated with a significant change in hemostatic parameters in a larger heterogenous population of healthy medium to large breed dogs administered 1 capsule of YB orally twice daily for 1 week. The secondary objective was to assess if there were any side effects attributed to YB administration.

Materials and Methods

The study protocol was approved by the Institutional Animal Care and Use Committee. Informed client consent was obtained in all cases. Dogs were deemed healthy based on an absence of clinical signs or previous medical conditions, and normal physical examination. Dogs were eligible if they were older than 12 months of age at the time of study initiation and if their body weight was >15 kg. Dogs were ineligible if they were receiving medications other than antiparasitics. The dogs were restrained and blood samples obtained from the jugular vein in all dogs. The blood was collected into blood tubes with 3.2% buffered sodium citrate solution in a 1:9 ratio following collection of a discard volume as per current recommendations.[14] All blood samples were stored at room temperature for 30 minutes prior to the TEG being performed by the principal investigator. Owners were then instructed to administer one 250 milligram capsule of YB[b] orally twice daily for 1 week, which equated to a dose range of 5.8–13.4 mg/kg. At the end of the study period, the owners were instructed to return with their dog and any remaining YB capsules. At this time, the remaining capsules were counted to verify compliance to the study protocol. The TEG was again repeated at this time by the same principal investigator in an identical manner described previously. Data collected included age, sex, breed, weight in kilograms, temperature, heart rate, respiratory rate, and side effects possibly attributed to YB administration and TEG parameters before and after YB administration.

In accordance with the current recommendations,[14,15] 1 milliliter of citrated whole blood was placed in a 1% kaolin vial, which was then inverted 5 times to ensure appropriate sample activation. Following activation, 340 μL of citrated whole blood and 20 μL of $CaCl_2$ were pipetted into a cuvette used in the TEG analyzer.[c] The TEG tracing was automatically stopped at 60 minutes after the MA was reached. The TEG results were generated by analyzer's software and included 10 variables (R, K, alpha, MA, G, CI, LY30, A30, LY60, and A60).[16] Quality assurance testing was completed every 8 hours of TEG testing. An owner questionnaire was provided at the end of the study (Day 7) for each patient enrolled in regards to adverse events, including lethargy, vomiting, diarrhea, lameness, change in appetite or behavioral changes.

The normality of data distribution was assessed using the Shapiro–Wilk test. Pre- and post-YB parameters were compared using Student's paired *t*-test. All tests were two-tailed, and a $P < 0.05$ was considered statistically significant. Results are reported as median and range unless otherwise indicated. Statistical analyses were performed using commercial statistical software.[d]

Results

Eighteen dogs were enrolled and completed the study. The enrolled breeds were mix breed ($n = 7$), Pit Bulls ($n = 4$), Labradors ($n = 3$), and 1 each of the following breeds: Doberman Pinscher, German Shorthaired Pointer, Golden Retriever, and German Shepherd Dog. There were 9 male neutered dogs, 4 male intact dogs, 3 female neutered dogs, and 2 female intact dogs. Median weight was 29.1 kg (range 18.6–40.6 kg) and

Table 1: Thromboelastography parameters pre- and posttreatment with 1 capsule of Yunnan Baiyao orally twice daily for 1 week in healthy dogs

Thromboelastography parameters (units)	Pretreatment mean (SD)	Posttreatment mean (SD)	Reference interval	*P*-value
R (min)	4.6 (4.6)	4.8 (3.9)	1.8–8.8	0.38
K (min)	2.1 (3.74)	1.9 (2.6)	1.3–5.7	0.24
Alpha (degrees)	58.45 (19.4)	62.05 (18)	36.8–74.6	0.13
MA (mm)	53.6 (14.1)	61.15 (8.4)	42.9–67.9	0.09
G (dynes/s)	5.95 (2.3)	7.85 (2.6)	3.2–9.6 $\times$ 10^3	**0.03**
CI	1.1 (12.5)	1.2 (5.3)	−3 to 3	0.72
LY30 (%)	4.45 (7.4)	0 (2.8)	NA	**<0.01**
A30 (mm)	47.5 (10.9)	59.65 (9.2)	NA	**<0.01**
LY60 (%)	8.75 (10)	0.85 (5.3)	NA	**< 0.001**
A60 (mm)	45.15 (11.6)	55.25 (10.2)	NA	**<0.01**

P-values that reached significance are bolded. Reference intervals for R, K, alpha, MA, G and CI adopted from Bauer et al.[17]
MA, maximal amplitude; CI, coagulation index; LY30 and LY60, percentage of clot lysis at 30 and 60 minutes after the MA has been reached; A30 and A60, the width of the TEG trace at its latest time point; NA, not applicable.

median age was 48 months (range 12–84 months). Median temperature on presentation was 37.7°C [100.8°F] (range 37.3–39.4°C [99.2–103.0°F] with a median heart rate of 110/min (range 66–170/min) and median respiratory rate of 30/min (range 24–60/min) with 5 dogs panting.

Baseline and recheck TEG parameters after 1 week of YB administration are shown in Table 1. Significant increases in G-value ($P = 0.03$), A30 ($P < 0.01$), and A60 ($P < 0.01$) values were noted as were significant decreases in LY30 ($P < 0.01$) and LY60 ($P < 0.001$). Of the 18 dogs in the study, 1 dog had mild self-limiting diarrhea that was reported on Day 7 of the study. The potential for the diarrhea being associated with YB administration could not be excluded, although this dog was the largest dog in the study (43 kg) and thus receiving the lowest dose of YB (5.8 mg/kg). All other dogs were reportedly normal and experienced no apparent side effects from the YB administration.

Discussion

This study identified that heathy medium to large breed dogs administered YB at 1 capsule orally twice daily for 1 week had TEG changes consistent with increased clot strength and reduced fibrinolytic activity. The administration of YB orally produced a significant increase in G and A30 and A60 values compared to baseline. The increase in these values may suggests that YB produced an increased in clot strength, although these parameters remained within the reference interval.

YB also apparently reduced fibrinolytic activity as evident by the significantly reduced values for LY30 and LY60 noted at 1 week recheck. Again, the changes in these parameters remained within the reference range as far as we could determine, although we did not establish a reference interval for any parameter. Interestingly, YB produced the TEG parameters that were expected but not produced by a study evaluating tranexamic acid use in dogs; in fact tranexamic acid produced the exact opposite effect as measured by TEG, increasing LY30 and LY60 and reducing A30 and A60.[18]

The current study found differences that are in contrast to results from the previous study of 8 Beagles treated with YB, which identified only a significant increase in R-value and no significant change in the G, A30, A60, LY30, and LY60 values.[a] The difference in results between the 2 studies may be due to the different assays employed (kaolin vs tissue factor as activators), patient populations, differences in sample size, duration of administration, variability of dose of YB administered, or variability of brand of YB administered. Further studies evaluating the hemostatic effects of YB in larger populations of various dog breeds should be considered.

Previous reports in YB in people and other noncanine veterinary species indicated that the mechanism of reduced clinical bleeding has enhanced platelet function.[1–5] No improvement was identified on TEG parameters that may be reflective of platelet function as there was an absence of significant change in K-value, alpha angle, and MA.[12,13] Platelet aggregometry or platelet mapping TEG would have been a more accurate option for evaluation but was not available at the authors' institution.[19] As such, the authors cannot rule out that YB may exert a similar beneficial improvement in platelet function as reported previously in addition to the findings reported here. Studies evaluating platelet function in dogs receiving YB may be warranted.

This study identified that YB appeared to be safe and well tolerated in healthy dogs weighing at least 15 kg at a dosage of 1 capsule orally twice daily. Only 1 of the 18 dogs had any possible side effects attributable to the

YB with one episode of diarrhea. However, as it developed on the last day of the study and resolved within 1 day, it is unclear if it was truly secondary to YB administration. Furthermore, this dog was the largest study participant (43 kg) and therefore was receiving the lowest dose of all dogs in the study (5.8 mg/kg).

There are several limitations to the study that warrant discussion. This is a small study of dogs that weighed >15 kg and thus there was still some weight variability between patients, which affected dosage of YB received. A placebo group was not included. The dogs included in the study did not have complete blood work, hemostatic function testing, or imaging studies performed prior to inclusion, so while they were clinically normal on history and physical examination, subclinical disease cannot be completely ruled out and its effect on hemostatic function and response to YB is unknown. Furthermore, another major limitation of this current study was that we did generate reference intervals for any TEG parameters and therefore could not evaluate whether baseline TEG parameters in this population of healthy dogs were within reference intervals. In addition, current recommendations are to evaluate hematocrit, fibrinogen concentration, and platelet counts of the animals undergoing TEG testing[16] as a previous study of TEG in animals have shown that there may be an inverse relationship between the hematocrit and MA, which can affect interpretation of results.[20] The authors of this study used the coagulation index to define hypercoagulable or hypocoagulable TEG tracings; however, at this time, there is insufficient evidence on how to standardize definitions for hyper- and hypocoagulability from TEG tracings.[16] The dose of YB evaluated here is anecdotal as there are no published doses available. It is not clear if the brand of YB used in this study is widely available and a recent study highlighted the variability of content of some Chinese herbal supplements.[21] Furthermore, as YB is a nutraceutical, it is not subject to the strict standards of quality control compared to that required of prescription medications. One of our findings was that YB may reduce fibrinolytic activity; however, as we did not establish a reference interval, we cannot determine the clinical significance of the difference identified. When our results are compared with the reference interval established for LY30 and LY60 using a tissue-factor assay, the changes may still be within the reference interval. In the current study, the authors performed a kaolin-activated TEG; however, TEG assays using tissue plasminogen activator may be more suitable for identifying changes in fibrinolytic activity.[16]

In conclusion, the present study suggests that this formulation of YB at a dose of 1 capsule orally twice daily may exerts a potentially beneficial hemostatic effect by increasing clot strength and reducing fibrinolytic activity in apparently healthy medium to large breed dogs. As the changes in TEG were still within reported reference intervals, the clinical significance of these changes is unknown. The administration of YB was apparently well tolerated with infrequent side effects. Larger prospective studies are warranted to evaluate these preliminary findings and the hemostatic and clinical benefit of YB administration in dogs with bleeding disorders.

Footnotes

[a] Lee A, Boysen S, Challhoub S, et al. Effects of Yunnan Baiyao on blood coagulation parameters in beagles measured using kaolin-activated thromboelastography. J Vet Emerg Crit Care 2015; 25:S24.
[b] Yunnan Baiyao Jiaonang, Yunnan Baiyao Group Co, Ltd, Kumming, China.
[c] Thrombelastograph Analyser 5000, at 37C, Haemoscope Corp, Niles, IL.
[d] SPSS 22.0 for Windows, Microsoft, Redmond, WA.

References

1. Chew EC. Effects of Yunnan Bai Yao on blood platelets: an ultrastructural study. Comp Med East West 1977; 5:169–175.
2. Chew EC. Yunnan Bai Yao-induced platelet release in suspensions of washed platelets. Comp Med East West 1977; 5:271–274.
3. Li NC, Pan BN, Wang HJ, et al. The effect of Yunnan Baiyao on reduction of intraoperative bleeding of the patients undergoing transurethral resection of prostate. Zhonghua Yi Xue Za Zhi 2007; 87:1017–1020.
4. Ogle CW, Dai S, Cho CH. The hemostatic effects of orally administered Yunnan Bai Yao in rats and rabbits. Comp Med East West 1977; 5:155–160.
5. Ogle CW, Dais S, Ma JC. The hemostatic effects of the Chinese herbal drug Yunnan Bai Yao: a pilot study. Am J Chin Med 1976; 4:147–152.
6. Ladas EJ, Karlik JB, Rooney D, et al. Topical Yunnan Baiyao administration as an adjunctive therapy for bleeding complications in adolescents with advanced cancer. Support Care Cancer 2012; 20:3379–3383.
7. Yang B, Xu ZQ, Zhang H, et al. The efficacy of Yunnan Baiyao on haemostasis and antiulcer: a systematic review and meta-analysis of randomized controlled trials. Int J Clin Exp Med 2014; 7:461–462.
8. Fan C, Song J, White CM. A comparison of the hemostatic effects of notoginseng and Yun Nan Bai Yao to placebo control. J Herb Pharmacother 2005; 5:1–5.
9. Ruggeri ZM. Von Willebrand factor, platelets and endothelial cell interactions. J Thromb Haemost 2003; 1:1602–1612.
10. Nurden AT, Caen JP. Specific roles for platelet surface glycoproteins in platelet function. Nature 1975; 255:720–722.
11. Shattil SJ. Signaling through platelet integrin alpha IIb beta 3: inside-out, outside-in, and sideways. Thromb Haemost 1999; 82: 318–325.
12. Donahue SM, Otto CM. Thromboelastography: a tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. J Vet Emerg Crit Care 2005; 15:9–16.
13. Mallet SV, Cox DJ. Thromboelastography. Br J Anaesth 1992; 6:307–313.
14. Flatland B, Koenigshof AM, Rozanski EA, et al. Systematic evaluation of evidence on veterinary viscoelastic testing Part 2: sample acquisition and handling. J Vet Emerg Crit Care 2014; 24: 30–36.
15. deLaforcade A, Goggs R, Winberg B. Systematic evaluation of evidence on veterinary viscoelastic testing Part 3: assay activation and test protocol. J Vet Emerg Crit Care 2014; 24:37–46.
16. Hanel RM, Chan DL, Conner B, et al. Systematic evaluation of evidence on veterinary viscoelastic testing Part 4: definitions and data reporting. J Vet Emerg Crit Care 2014; 24:47–56.
17. Bauer N, Eralp O, Moritz A. Establishment of reference intervals for kaolin-activated thromboelastography in dogs including an

assessment of the effects of sex and anticoagulant use. J Vet Diagn Invest 2009; 21:641–648.
18. Kelmer E, Segev G, Papshvilli V, et al. Effects of intravenous administration of tranexamic acid on hematological, hemostatic and thromboelastographic analytes in healthy adult dogs. J Vet Emerg Crit Care 2015; 25:495–501.
19. Jandrey KE. Assessment of platelet function. J Vet Emerg Crit Care 2012; 22:81–98.
20. Brooks AC, Guillaumin J, Cooper ES, et al. Effects of hematocrit and red blood cell-independent viscosity on canine thromboelastographic tracings. Transfusion 2014; 54:727–734.
21. Shmalberg J, Hill RC, Scott KC. Nutrient and metal analyses of Chinese herbal products marketed for veterinary use. J Anim Physiol Anim Nutr 2013; 97:305–314.