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Full Length Article

Effects of Yunnan Baiyao on blood coagulation parameters in beagles measured using kaolin activated thromboelastography and more traditional methods

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ABSTRACT

Yunnan Baiyao is anecdotally widely used in veterinary medicine for its pro-coagulation properties. There are no studies determining its effect on clotting tests. The purpose of this study was to determine the effect of oral Yunnan Baiyao (YB) on hemostasis by measuring coagulation via kaolin activated citrated whole blood thromboelastography (TEG), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and d-dimers. The study was a prospective, randomized controlled crossover trial. Eight healthy University of Calgary owned beagles were treated orally with either Yunnan Baiyao or placebo every 12 h for 5 treatments. Blood was collected immediately before treatment, 2 h after the last treatment, and 24 h after the last treatment. TEG analysis was run 30 ± 5 min after blood collection. All other coagulation analyses were sent to a reference laboratory for further analysis. No treatment adverse effects were observed. Oral YB increased R-time significantly within the YB group, but the increase was still within canine reference ranges. No other changes were observed. Oral YB at the dosage and frequency administered in this study did not produce any significant improvement in hemostatic parameters. There is a need for further research and scientific evidence for YB use and dosage.

1. Introduction

Yunnan Baiyao (YB) is a Chinese herbal remedy which is thought to promote blood clotting and hemostasis. Despite the limited knowledge regarding its mechanism of action, safety, and efficacy, YB is currently used in human and veterinary medicine as an adjunct therapy to minimize bleeding during surgery or to help with hemorrhagic disorders [1,2]. In addition to its anti-hemorrhagic properties, YB may also have anti-inflammatory effects [1,3,4].

Human studies have demonstrated clinical hemostatic efficacy of pre-operative oral YB administration, with observed decreased blood loss during surgery [5,6]. Oral YB has been shown to decrease buccal mucosal bleeding times (BMBT) in rats and rabbits, as well as promote platelet constituent release in rats [7–9]. YB has also been investigated in rats for topical administration on surgically transected tails, with a significant decrease in total bleeding time being observed [8].

While studies evaluating the efficacy of YB in different species are emerging, there are currently no studies evaluating its anti-hemorrhagic effects in dogs despite its anecdotally widespread use [1–3,7]. This study evaluates the effect of oral YB on hemostasis of 8 healthy beagles, as measured by thromboelastography (TEG), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and d-dimers. It is our hypothesis that the administration of oral YB will result in a hypercoagulable TEG tracing with evidence of decreased fibrinolysis compared to controls, and will not impact the traditional coagulation parameters PT, aPTT, d-dimers, or fibrinogen.

2. Materials and methods

Animal care protocols were approved for this study.[1] Eight healthy beagles were included – 2 females and 6 males. Dogs ranged

Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time; TEG, thromboelastography; YB, Yunnan Baiyao.

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from 4 to 7 years of age (median age 6 years), and were all between 9 and 14 kg (19.8–30.9 lb) (average weight 11.5 kg/25.4 lb) in weight. Dogs were housed in two identical rooms according to group, and fed the same diet according to weight. Beagles were randomly (block 1:1) assigned to two groups; control vs YB. Researchers were blinded to group assignments until data analysis was completed. Inclusion criteria for this study were: 1) a normal physical exam, 2) a normal complete cell blood count (CBC) and biochemistry panel, 3) a normal coagulation profile which included TEG, PT, aPTT, fibrinogen, d-dimers, and platelet count, and 4) no medications or supplements for at least 30 days prior to study enrollment. Animals were evaluated daily by physical exam, and treated every 12 h for 5 treatments with either 2× 250 mg capsules of YB inside pill pockets (YB) based on manufacturer recommendations or 2 empty pill pockets (control).[2] Blood collection occurred immediately before starting treatment, 2 h and 24 h after the last treatment.

Six mL of blood was collected via jugular venipuncture using 20 gauge 1 inch needles, 6 cc syringes and alternating jugular veins for each collection [9]. Only a single venipuncture per jugular vein was attempted for blood sampling, otherwise the other jugular vein was used. Blood was immediately transferred into two 3.2% sodium citrate vacuum tubes – 3 mL per tube, gently inverted 5 times and kept at room temperature. One citrated blood tube was used for TEG analysis, and the other sent to referral laboratory[3] for coagulation profile (PT, aPTT, fibrinogen, d-dimers) analysis in the same day. TEG analysis was started 30 ± 5 min after venipuncture. TEG analysis was performed using Thromboelastograph Analyzers[4] at 37 °C (98.6° F). All TEG analyses were performed in duplicate simultaneously.

A ten day washout period was allowed, after which a crossover study was performed in an identical manner.

2.1. TEG analysis

One mL of citrated whole blood was transferred into room temperature standardized kaolin tubes[5] containing 40 μL of kaolin and inverted 5 times to mix thoroughly, after which 340 μL of the kaolin activated blood was immediately added to non-heparinized TEG cups[6] containing 20 μL of room temperature 0.2 M calcium chloride and the TEG analysis started. TEG was run until the R, K, angle, MA, G, Ly30 and Ly 60 values were recorded. E-tests and Level 1 and Level 2 controls were run as per Haemontics package insert instructions the morning of any analysis and were within normal range during this study [10].

2.2. Statistical analysis

Duplicate TEG results were averaged and used as a single data point. Normalcy of results were determined using the D'agostino and Pearson omnibus normality test. Comparison of results was performed using 1-way repeated measures ANOVAs to look for differences in coagulation parameters within groups while 2-way repeated measures ANOVAs were used to look for differences in coagulation parameters between groups. All analyses were performed using statistical software package[7], with values being considered significant at $P < 0.05$.

[2] Greenies Pill Pockets. The Nutro Company, Franklin, TN.
[3] Antech Canada Diagnostic Laboratory. Calgary, AB, Canada.
[4] TEG 5000 Thromboelastograph Hemostasis Analyzer System. Haemontics Corp, Niles, IL.
[5] TEG 5000 Kaolin. Haemontics Corp, Niles, IL.
[6] TEG 5000 Non-heparinized TEG Cups. Haemontics Corp. Niles, IL.
[7] Prism, version 6.05, GraphPad Software Inc, San Diego, CA.

3. Results

All 8 dogs completed both the initial and crossover phases of the study without complication. No adverse effects of treatment were noted based on physical examination at any point throughout the study. Dosage ranged from 55.6 mg/kg to 35.7 mg/kg every 12 h for 5 days based on body weights. Based on an average dog weight of 11.5 kg, the average dosage was 43.5 mg/kg every 12 h for 5 days. The results of the control and YB groups are shown in Table 1.

The R time at 2 and 24 h post treatment in the YB group was statistically prolonged compared to baseline (Fig. 1) ($p < 0.05$). No statistically significant changes were noted in the 2-way ANOVA comparing R time between YB and control groups. No significant changes in TEG values K, MA, G, α-angle, Ly30 or Ly60 were detected within or between groups.

No significant changes were detected in PT, aPTT, fibrinogen, d-dimers within or between groups. The 95% confidence intervals (CI) for mean differences between each parameter are presented in Table 1. Although ranges of values in these CI's are variable, they each include the zero difference value.

4. Discussion

Human and lab animal studies suggest an appreciable clinical effect of YB, with observed decreased intra-operative blood loss, increased platelet function observed via electron microscopy, and faster clotting times as measured by BMBTs [1,2,7,9]. The results of the present study do not align with these findings as our study failed to show any clinically significant changes in any of the coagulation parameters assessed in our beagle population. There was a statistically significant increase in the R-time following YB administration in the treatment group, which indicates an increase in the initial time to clot formation. This may suggest a more hypocoagulable state, which is in contrast to the literature; however, the result is not likely clinically significant as the elevated R-time was still within normal canine reference ranges {R = 1.8–8.6 min} [11]. Additionally, the two-way ANOVA comparing the YB group to the control did not reveal any significance. The 95% CI's of the mean difference between control and YB groups reveal varied ranges, but all include the zero difference value and in no instance support that a clinically important difference would be found at the dose range used in this study even if statistical significance is reached with a larger sample size. Future studies therefore should consider investigating increased dose and/or increased frequency of YB administration in dogs.

This is the first study to evaluate the effect of oral YB using TEG. Given the dose and treatment regimen used in this study, oral YB had no significant effect on blood coagulation parameters as measured by PT, aPTT, fibrinogen, d-dimers, or TEG K, MA, α-angle, G, Ly30, or Ly60. It is interesting to note that a previous study observed clinical effects of oral YB and decreased intra-operative surgical blood loss in humans, but did not observe significant differences in PT, aPTT, fibrinogen, or d-dimer values [1]. This suggests that a lack of change in coagulation and fibrinolytic tests does not necessarily imply a lack of clinical effect, or that the effect of YB is a result of primary hemostasis as opposed to secondary hemostasis or fibrinolysis. There is evidence to suggest the hemostatic effects of YB are due to an effect on platelet function and primary hemostatic mechanisms as suggested by lab animal studies evaluating BMBTs (2009) [7,9]. Normal canine BMBTs have previously been measured with wide variations in results. Normal values range from 109 to 285 s, with inter and intra observer repeatability being ±113 and ±126 s respectively [12]. BMBT was

Table 1
Mean (±SD), confidence intervals (95%) of mean differences between treated (YB) and control groups (n = 8 dogs/group) for thromboelastography parameters, PT, PTT and Fibrinogen.

Parameter	Time point	Treatment (YB) Mean ± SD	Control Mean ± SD	Mean difference	95% CI of difference	P value
R value (min)	Base line	1.99 ± 0.55	2.62 ± 0.86	−0.62	−1.4 to 0.19	0.19
	2 h	2.96 ± 0.92	2.37 ± 0.47	0.59	−0.22 to 1.4	0.23
	24 h	3.06 ± 0.42	2.63 ± 0.54	0.44	−0.38 to 1.25	0.57
Alpha angle (°)	Base line	72.49 ± 3.67	69.59 ± 6.78	2.9	−2.3 to 8.2	0.52
	2 h	71.58 ± 3.05	72.54 ± 3.20	−0.96	−6.2 to 4.3	>0.99
	24 h	71.14 ± 3.28	71.87 ± 4.08	−0.72	−6.0 to 4.5	>0.99
K value (min)	Base line	1.36 ± 0.37	1.35 ± 0.34	0.01	−0.37 to 0.40	>0.99
	2 h	1.31 ± 0.28	1.27 ± 0.39	0.04	−0.34 to 0.43	>0.99
	24 h	1.28 ± 0.17	1.30 ± 0.36	−0.02	−0.41 to 0.37	>0.99
MA (mm)	Base line	57.76 ± 3.79	59.79 ± 5.80	−1.9	−7.9 to 4.1	>0.99
	2 h	57.68 ± 2.35	57.28 ± 4.99	0.41	−5.6 to 6.4	>0.99
	24 h	59.37 ± 4.94	58.76 ± 6.01	0.61	−5.4 to 6.6	>0.99
G value (dyn/s)	Base line	7.00 ± 1.03	7.75 ± 1.84	−0.75	−1.2 to 2.7	>0.99
	2 h	6.90 ± 0.68	6.90 ± 1.44	0.004	−1.9 to 1.9	>0.99
	24 h	7.53 ± 1.48	7.44 ± 2.19	0.088	−2.0 to 1.8	>0.99
Ly30 (%)	Base line	0.76 ± 0.75	1.43 ± 2.23	−0.67	−2.8 to 1.4	>0.99
	2 h	2.15 ± 1.98	2.02 ± 2.35	0.13	−2.0 to 2.2	>0.99
	24 h	1.31 ± 1.27	0.55 ± 0.73	0.76	−1.3 to 2.9	>0.99
Ly60 (%)	Base line	3.94 ± 2.79	5.15 ± 5.38	−1.2	−6.2 to 3.8	>0.99
	2 h	5.69 ± 3.90	6.85 ± 5.25	−1.2	−6.1 to 3.8	>0.99
	24 h	4.56 ± 2.42	3.64 ± 3.16	0.91	−4.1 to 5.9	>0.99
PT (s)	Base line	8.41 ± 0.73	8.04 ± 0.58	0.38	−0.70 to 1.4	>0.99
	2 h	7.49 ± 0.84	7.61 ± 0.69	−0.12	−1.2 to 0.95	>0.99
	24 h	6.93 ± 1.27	6.70 ± 0.89	0.23	−0.85 to 1.3	>0.99
PTT (s)	Base line	10.93 ± 1.26	10.48 ± 0.51	0.45	−0.43 to 1.3	0.63
	2 h	10.69 ± 0.44	10.58 ± 0.45	0.11	−0.77 to 0.99	>0.99
	24 h	10.78 ± 0.79	10.48 ± 0.50	0.30	−0.58 to 1.2	>0.99
Fibrinogen (g/L)	Base line	1.16 ± 0.28	1.37 ± 0.34	−0.21	−0.53 to 0.12	0.37
	2 h	1.51 ± 0.21	1.43 ± 0.21	0.079	−0.25 to 0.40	>0.99
	24 h	1.39 ± 0.27	1.46 ± 0.24	−0.076	−0.40 to 0.25	>0.99

CI, confidence interval; SD, standard deviation; YB, Yunnan Baiyao; R, reaction time; α-angle, angle between R and K; K, time from 2 to 20 mm amplitude; MA, maximum amplitude; G, shear elastic modulus strength; LY30, percent clot lysis at 30 min; LY60, percent clot lysis at 60 min; PT, prothrombim time; PPT, partial thromboplastin time.

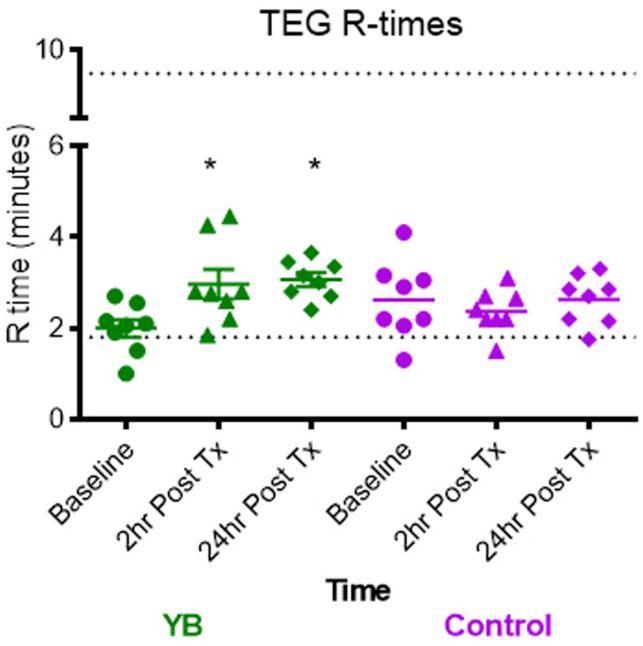

Fig. 1. R-times from the YB and control groups. Oral YB treatment resulted in an increase in R-time. These values are still within established reference ranges as illustrated by the dotted horizontal lines. Asterisk indicates significance compared to baseline values within groups from a 1-way ANOVA ($P < 0.05$).

not evaluated in this study because of the inconsistency of results as well as variation created by operator experience [13].

Thromboelastography assesses the global coagulation process including some degree of platelet function as measured by MA. However, TEG may not be sensitive enough to detect any effect on intrinsic platelet components that would subsequently alter platelet activation, or on any extrinsic components such as von-Willebrands Factor (vWF). Platelet activation can be more specifically measured through TEG platelet mapping (TEG-MP) [14]. TEG-MP has been used to successfully detect platelet activation changes in hypercoagulable states in dogs, and could be useful in determining whether alterations in platelet activation are involved [14]. Plasma vWF levels and functionality could be further evaluated to determine any potential effects on this protein by YB [15]. Determining the mechanism of action of YB would allow for targeted use in specific disorders should YB prove to be effective.

To our knowledge, prior to our study the effects of YB on the fibrinolytic process has not been assessed. The lack of change in the TEG Ly30 and Ly60 values both within and between groups in the present study suggests that either YB does not affect the fibrinolytic process, or given the parameters of this study the effect was not detectable.

It is known that pre-analytical variation can have significant effects on TEG results. Such variation includes sampling technique, blood storage and handling time [16]. Pre-analytical variation was minimized in this study by having the same person perform all the blood collections, handling, transport, and tests in order to keep the technique and timing consistent. Activators such as kaolin or tissue factor (TF) help standardized clotting initiation and decrease test sensitivity to pre-analytical variation [17]. Kaolin was selected for this study because normal reference ranges have already been established in dogs, and its use in research is well established with standardized protocols [11,18].

It should be noted that the TEG analysis in this study was performed at 37 °C (98.6° F), while physiologic temperature in canine is typically closer to 39 °C (102.2° F), potentially altering clot kinetics when compared to physiologic states. However, the majority of

TEG studies in veterinary medicine are performed at 37 °C (98.6° F), and established reference ranges for TEG values using kaolin activated citrated whole canine blood were determined at 37 °C (98.6° F) as well [11,17].

One of the biggest limitations of the current study was the small sample size. A sample size of n = 8 provides limited detection power and as such small changes in data could easily be missed. Although studies with a larger sample size might detect statistically significant differences, as discussed above regarding CI's, it is unlikely the difference would be clinically significant at the dosage used in this study.

Without any prior studies evaluating the efficacy of oral YB on hemostasis in dogs, the treatment schedule in this study was based on human trials which observed clinical effects of oral YB [1]. The optimization of this schedule remains undetermined in dogs. The sampling times were also based on studies in other species – rabbits and rats, which observed significant hemostatic effects of oral YB measured via BMBT starting 30 min post-administration and persisting till at least 4 h post administration [9]. The duration of efficacy has not yet been established in dogs, and extrapolation from other species may have lead to sampling times that did not reflect peak hemostatic effects of YB due to species differences in the pharmacodynamics of YB. The dosage requirements in dogs also remain undetermined. The manufacturer of YB only provides dosages for human use. The dosage utilized in the present study was selected from an article with suggested YB dosage in animals [19]. However, the dose ranges of YB are poorly defined and widely variable. Without studies evaluating the hemostatic efficacy of YB, it is impossible to say whether this dose is effective or not. Dosing is often to effect and may vary depending on the cause of the bleeding, reinforcing the fact that little is known about the efficacy and potency of YB [19]. Commonly used doses of YB for hemorrhage control are higher and more frequent than what was used in the present study. Further studies should also therefore examine a higher dose of YB, increased dosage frequency, as well as the timing of blood sampling.

As a nutraceutical, there is little regulation on product quality of YB [20]. As a result inconsistencies in product quality between packages and suppliers remains high, and may account for variability between and within studies evaluating the hemostatic efficacy of YB. Determining the consistency of the YB formulation would be useful to determine how standardized treatments are solely based off of a mg/kg dosage.

While no adverse effects of oral YB were noted based on physical examination is consistent with previous studies, it is possible that changes undetectable by physical examination alone may be occurring [1,3,4,8,9]. Post treatment CBC and biochemistry panels would provide more information about potential side effects of oral YB administration.

5. Conclusions

Oral YB administered at the dosage used in this study (500 mg per 5–15 kg dog PO BID) for 5 treatments had no statistically significant effect on TEG, PT, aPTT, fibrinogen, or d-dimers at 2 or 24 h post-treatment. There was a statistically significant increase in TEG R-time within the YB group, which was unlikely of clinical significance. Oral YB appears to be safe at the dosages administered based on physical examination. The mechanism of action of YB on hemostasis remains unknown.

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