Hemorrhage is frequently encountered in equine practice. Life-threatening blood loss can occur as a result of trauma, vascular infiltrative disease, or systemic coagulopathy. Case fatality rates are high for horses with acute hemorrhage from surgically inaccessible sites (eg, internal carotid artery or middle uterine artery). This is particularly true for periparturient rupture of the uterine artery whereby hemorrhage into the uterus and abdomen leads to rapid hypovolemic shock and death, which results in the loss of valuable broodmares and a number of orphan foals every year. These animals typically are treated empirically by use of a combination of fluid therapy and a variety of hemostatic agents. The traditional Chinese herbal formula Yunnan Baiyao has been used by owners, trainers, and veterinarians to manage acute hemorrhage in horses. However, mechanistic insights into the hemostatic properties of Yunnan Baiyao are lacking, which hinders design of treatment studies to improve outcomes for uterine artery rupture and other acute hemorrhagic disorders of horses.

Yunnan Baiyao was formulated in 1902 in China and has been designated as a class 1 protected traditional Chinese medicine with associated restrictions on its production. Historically, Yunnan Baiyao was carried by Chinese soldiers in World War II and later by the Viet Cong for control of hemorrhage on the battlefield. The wide clinical use of Yunnan Baiyao as a hemostatic agent in China is comparable to that of penicillin as a routine antibacterial agent in the United States. Yunnan Baiyao is a powder contain-
ing a combination of various indigenous medicinal plants, one of which is *Panax pseudoginseng* (also called notoginseng). Yunnan Baiyao's active constituents have been identified through high-performance liquid chromatographic techniques. An evaluation of 27 commercial preparations revealed a consistent batch-to-batch composition of 2 major ginsenoside saponins (ginsenoside Rg1 and Rb1), which are thought to provide at least some of the hemostatic properties. In vitro studies have revealed that various saponins are capable of inducing a strong activation response in human platelets. Moreover, preliminary clinical trials of humans have indicated some benefit of Yunnan Baiyao for reducing intraoperative blood loss. However, the authors are aware of only 2 studies in which the effects of Yunnan Baiyao in horses have been evaluated. A decrease in template bleeding time was detected for 6 anesthetized ponies after oral administration of Yunnan Baiyao; however, a study of exercise-induced pulmonary hemorrhage in 5 Thoroughbreds after treadmill exercise found no clinical benefit.

Because of the paucity of evidence, coupled with the widespread use of Yunnan Baiyao, the purpose of the study reported here was to investigate the hemostatic actions of Yunnan Baiyao in healthy horses. We hypothesized that Yunnan Baiyao would exert a hemostatic effect mediated through enhanced platelet reactivity or increased fibrin clot stability (or both). A comprehensive panel of assays was used to evaluate a wide variety of hemostatic pathways and proteins encompassing platelet-activation responses and variables of fibrin formation and fibrinolysis in horses treated by oral administration of Yunnan Baiyao and a placebo.

**Materials and Methods**

**Animals**

Twelve healthy adult horses (10 mares and 2 geldings; 5 Thoroughbreds, 4 warmbloods, 2 Holsteiners, and 1 Oldenburg) from a teaching facility were included in the study. Median age of horses was 15.5 years (range, 11 to 18 years), and median body weight was 543 kg (range, 500 to 663 kg). Horses were considered healthy on the basis of clinical history, physical examination findings, and results of a CBC and hematologic biochemical analyses. The horses had not received any medication or supplement-type products for at least 3 weeks prior to the study. Horses were transported from a paddock to box stalls and allowed to acclimate for 24 hours before start of the study. The study protocol was approved by an institutional animal care and use committee.

**Study design and treatments**

An in vivo treatment trial and laboratory assays were performed between January and November 2014 at the Cornell University College of Veterinary Medicine. The study was performed as a randomized blinded placebo-controlled crossover study with a 4-week washout period between subsequent treatments to allow for complete turnover of platelets and hemostatic proteins. Horses were examined and vital signs recorded after the 24-hour turnover period to the box stalls and before administration of each treatment during the study.

Compounded pastes that contained Yunnan Baiyao (15 mg/kg) or a visually indistinguishable placebo (cornstarch powder) were administered to horses via dosing syringe at 12-hour intervals for 3 days. Treatment order was assigned by use of a randomization software application, and the investigators were unaware of treatment assignment until all laboratory analyses were completed.

**Blood sample collection and processing**

Blood samples were collected before the start of treatment (time 0; baseline) and 24 and 72 hours after start of treatment administration. Blood samples were collected by jugular venipuncture into evacuated glass anticoagulant tubes that contained EDTA or heparin and directly into 35-mL syringes that contained one-tenth volume of 3.8% sodium citrate. The EDTA-anticoagulated WB was used for CBCs, heparinized WB was used for biochemical panels, and citrated WB was used in assays or processed into components as described elsewhere. In brief, PRP (≥150,000 platelets/µL) was prepared by centrifugation of WB at 450 X g for 5 minutes; platelet- and leukocyte-rich plasma was harvested from WB that had been allowed to sit undisturbed for 20 minutes; and PPP was isolated after centrifugation of WB at 2,500 X g for 15 minutes. Samples of WB, PRP, and platelet- and leukocyte-rich plasma were prepared and analyzed within 1 hour after blood collection; aliquots of PPP were stored at -50°C for subsequent batch testing.

**Laboratory analyses**

The CBCs and biochemical panels were performed at a university clinical pathology laboratory. Platelet adhesion and aggregation in WB were measured as closure time by use of a tabletop platelet-function analyzer and the manufacturer’s reaction cartridges that contained ADP and collagen. Light transmission aggregometry was performed by use of PRP, with no adjustment to a standard platelet count. Maximal percentage aggregation was measured in a multichannel aggregometer as the response to stimulation with ADP at final concentrations of 0.6 and 1.25 µM and collagen at final concentrations of 6 and 12 µg/mL. Reactions were conducted at 37°C in a final volume of 250 µL, with stirring at 1,200 cycles/min for the 6-minute observation period.

Flow cytometry reactions used platelet- and leukocyte-rich plasma samples dual-labeled with an antibody directed against the constitutive platelet antigen (CD61) and 2 alternate activation markers to detect expression of platelet P-selectin (CD62P) or externalization of phosphatidylserine (annexin V).
The subset of events labeled with CD61 that were below the first decade of the logarithmic forward-scatter scale was classified as small platelets and PMP.

Platelet-activation status and PMP were measured in samples with no exogenous stimulation and after ex vivo addition of thrombin (0.1 U/mL), in accordance with conditions described elsewhere. Acquisition was set for 7,500 platelet events defined by forward- and side-scatter properties. Data were analyzed to measure the percentages of CD61-positive events labeled with various activation markers and the percentage of PMP. Unstimulated platelet- and leukocyte-rich plasma samples labeled with CD61 were also collected by use of a separate acquisition template and used to differentiate leukocyte subpopulations (neutrophils, monocytes, and lymphocytes) on the basis of forward- and side-scatter properties.

The platelet- and leukocyte-rich samples were collected to obtain 10,000 total events and analyzed to determine the percentage of CD61-labeled events (leukocyte-associated platelets) within each leukocyte gate.

Thrombelastography was performed with a viscoelastic monitor by use of a human tissue-factor reagent (dilution, 1:100) to trigger coagulation in WB, as previously described. Reaction time (time to initial clot formation [R]), clotting time (K), rate of clot formation (α angle), and the measure of the final clot strength (maximal amplitude [MA]) were determined by the instrument software. In addition, a modified TEG assay was performed to evaluate fibrinolysis in PPP samples. The assay was configured in a total volume of 340 µL that contained 320 µL of PPP, 10 µL of tissue factor reagent (dilution, 1:50), and 10 µL of recombinant human tPA diluted in a saline (0.9% NaCl) solution-bovine serum albumin buffer. Paired lysis reactions were performed in PPP with a final concentration of 250 or 500 U of tPA/mL to induce minimal or maximal clot lysis within 60 minutes. For fibrinolysis TEG assay, percentage clot lysis at 30 and 60 minutes after MA and clot lysis time were recorded in addition to R, K, α angle, and MA. All TEG assays were performed as a singlicate analysis.

Coagulation panels (consisting of aPTT, PT, and Clauss fibrinogen determinations) were performed with an automated coagulation analyzer, as described elsewhere. Plasma concentration of vWF:Ag was measured by use of a quantitative multispecies ELISA. Activity of plasma vWF was measured by use of a vWF:RCo assay that was based on the ability of VWF high-molecular-weight multimers to agglutinate platelets in the presence of the antimicrobial ristocetin. Agglutination reactions were measured in an aggregometer by use of a commercial vWF:RCo kit that contained lyophilized human platelets, ristocetin, and a human calibrated vWF standard. Samples were assayed at a dilution of 1:4 (rather than 1:2) to enhance the agglutination reaction in equine plasma; results were calculated as the percentage agglutination compared with that of pooled equine plasma.

In vitro bioassay

Procoagulant activity of the lot of Yunnan Baiyao used in the study was evaluated in an in vitro clotting time test, which was modified from a method described elsewhere. Clotting times were compared for aliquots of pooled normal human plasma and pooled equine plasma combined with equal volumes of a buffer (50 mM Tris-HCl [pH, 7.4]) as a diluent control, or 5.0-mg/mL suspensions in buffer of Yunnan Baiyao as the active treatment, or 5.0-mg/mL suspensions in buffer of gelatin powder as an inert, negative control substance. Coagulation was initiated through activation of factor X in the common pathway by the addition of a snake venom reagent and recalcification.

Statistical analysis

Sample size calculation for the study was based on an observed response in humans to Yunnan Baiyao used as a preoperative prophylactic agent, which results in a decrease in blood loss of approximately 20%. It was determined that a sample size of 12 horses (2-sided test; α = 0.05) would yield a 90% chance of detecting an increase of ≥17% in the hemostatic response for the Yunnan Baiyao treatment. Shapiro-Wilk tests for goodness of fit for a Gaussian distribution indicated that at least 12 of 30 percentage changes had non-Gaussian distributions; therefore, data were analyzed nonparametrically. For each variable, descriptive statistics were summarized as median and range (ie, minimum and maximum) values.

To minimize the likelihood of obtaining false-positive results as a consequence of multiple comparisons, the number of statistical comparisons was reduced by use of the following strategy: only samples obtained at 0 and 72 hours were analyzed. Twenty-eight variables of interest were subdivided into categories on the basis of their role in hemostasis as follows: primary hemostasis (platelet count, closure time, vWF:Ag concentration, activity determined by use of the vWF:RCo assay, ratio of vWF:Ag concentration to vWF activity determined by use of the vWF:RCo assay, maximal aggregation in response to ADP and collagen, resting and stimulated expression of P-selectin and phosphatidyserine and release of PMP, and platelet-leukocyte aggregate formation); coagulation (aPTT, PT, and fibrinogen concentration); WB TEG (Hct, R, K, α angle, MA, and percentage clot lysis at 60 minutes after MA), and fibrinolysis TEG with 500 U of tPA/mL (R, K, MA, percentage clot lysis at 60 minutes after MA, and clot lysis time). Within
Within-horse effects of Yunnan Baiyao were compared with results for the placebo. The Wilcoxon signed rank test was used to compare the percentage change from time 0 in the placebo treatment versus the Yunnan Baiyao treatment for each of the selected 15 variables as described previously. Bonferroni corrections (for the overall value for significance of \( P = 0.05 \)) were used to set critical \( P \) values within each group of multiple comparisons. All statistical analyses were performed by use of commercially available software.

Preliminary analyses revealed that 1 horse had an undetectable baseline value for vWF activity determined by use of the vWF:RCo assay and a markedly elevated (>300%) vWF:Ag concentration, which suggested a variant vWF protein. This horse had no evidence of abnormal hemorrhage before or during the study and had no abnormalities in baseline closure times and aggregation; however, data for this horse were excluded from treatment comparisons involving vWF because of these unexplained vWF abnormalities.

### Results

#### Animals

All horses remained healthy throughout the study. There were no relevant abnormalities in vi-

<p>| Table 1—Median (range) values for hemostatic variables in samples obtained before (time 0) and 72 hours after the start of treatment for 12 horses receiving Yunnan Baiyao (15 mg/kg) and a placebo orally at 12-hour intervals for 3 days in a crossover study; there was a 4-week washout period between treatments. |</p>
<table>
<thead>
<tr>
<th>Variable</th>
<th>Yunnan Baiyao</th>
<th>Placebo</th>
<th></th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary hemostasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets (X 10^3 cells/µL)</td>
<td>126 (99–170)</td>
<td>132 (104–179)</td>
<td>112 (66–174)</td>
<td>131 (71–196)</td>
</tr>
<tr>
<td>Closure time (s)</td>
<td>82 (56–108)</td>
<td>96 (70–173)</td>
<td>85 (70–120)</td>
<td>84 (66–112)</td>
</tr>
<tr>
<td>vWF:Ag (%) †</td>
<td>104 (76–117)</td>
<td>101 (83–126)</td>
<td>105 (74–129)</td>
<td>103 (79–138)</td>
</tr>
<tr>
<td>vWF:Ag to vWF:RCo †</td>
<td>89 (64–113)</td>
<td>113 (57–139)</td>
<td>106 (63–160)</td>
<td>108 (77–171)</td>
</tr>
<tr>
<td>ADPMA (%)</td>
<td>50 (31–63)</td>
<td>46 (29–65)</td>
<td>46 (37–68)</td>
<td>52 (27–73)</td>
</tr>
<tr>
<td>PselTH (%)</td>
<td>7.8 (4.4–26.0)</td>
<td>5.0 (8.8–20.0)</td>
<td>5.6 (0.7–42.0)</td>
<td>11.0 (3.1–74)</td>
</tr>
<tr>
<td>ASTH (%)</td>
<td>2.6 (1.1–4.9)</td>
<td>2.4 (1.3–4.0)</td>
<td>2.5 (1.2–8.3)</td>
<td>2.4 (1.4–7.5)</td>
</tr>
<tr>
<td>PMPTH (%)</td>
<td>3.0 (1.8–11.0)</td>
<td>3.7 (2.5–7.1)</td>
<td>3.2 (2.1–5.1)</td>
<td>3.6 (1.6–5.9)</td>
</tr>
<tr>
<td>PLeuk (%)</td>
<td>4.6 (1.7–16.0)</td>
<td>3.7 (1.9–18.0)</td>
<td>4.6 (1.9–17.0)</td>
<td>3.7 (2.5–22.0)</td>
</tr>
<tr>
<td><strong>Secondary hemostasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>45 (44–49)</td>
<td>47 (45–51)</td>
<td>46 (40–49)</td>
<td>47 (43–50)</td>
</tr>
<tr>
<td>PT (s)</td>
<td>13 (12–14)</td>
<td>13 (12–14)</td>
<td>13 (12–14)</td>
<td>13 (12–14)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>334 (264–409)</td>
<td>332 (283–429)</td>
<td>332 (285–384)</td>
<td>353 (287–384)</td>
</tr>
<tr>
<td><strong>Fibrinolysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tPA CLT (min)</td>
<td>5.9 (3.8–12.0)</td>
<td>4.7 (1.6–61.0)</td>
<td>7.4 (3.0–61.0)</td>
<td>6.0 (2.6–28.0)</td>
</tr>
<tr>
<td>WB TEG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>40 (32–49)</td>
<td>40 (34–44)</td>
<td>39 (33–49)</td>
<td>40 (33–45)</td>
</tr>
<tr>
<td>α angle (“)</td>
<td>18 (12–62)</td>
<td>22 (12–29)</td>
<td>24 (14–62)</td>
<td>19 (13–39)</td>
</tr>
</tbody>
</table>

*Results of Wilcoxon signed rank test for the comparison between 0 and 72 hours for each treatment group; no significant differences between treatments were detected for any variable (Bonferroni corrected \( \alpha \) was as follows: primary hemostasis variables, 0.0055; secondary hemostasis variables, 0.017; fibrinolysis, 0.05; and WB TEG, 0.025). † Represents results for only 11 horses. 

α Angle = Rate of clot formation. ASTH = Thrombin-stimulated phosphatidyserine externalization. ADPMA = Aggregation response (maximal amplitude [MA]) to 1.2 µM ADP. PLeuk = Platelet-leukocyte aggregates. PMPTH = Thrombin-stimulated release of PMP. PselTH = Thrombin-stimulated P-selectin expression. tPA CLT = tPA (500 µL/mL)-induced clot lysis time. vWF:Ag to vWF:RCo = Ratio of vWF:Ag concentration to vWF activity determined by use of the vWF:RCo assay.

... each category, Spearman rank correlations were performed on the measurement at time 0 for the placebo treatment to identify and eliminate redundant variables. When the rank correlation had a value of \( P \leq 0.05 \), then one of the paired variables was selected for removal from the main analyses. In addition, the decision was made a priori to analyze only the single lysis variable of fibrinolysis TEG clot lysis time. Of the 28 original variables, a subset of 15 variables was compared between the Yunnan Baiyao and placebo treatments: platelet count, Hct, closure time, vWF:Ag concentration, ratio of vWF:Ag concentration to vWF activity determined by use of the vWF:RCo, maximal aggregation to ADP, stimulated expression of P-selectin and phosphatidyserine, thrombin-stimulated release of PMP, platelet-leukocyte aggregates, aPTT, PT, fibrinogen concentration, WB TEG α angle, and fibrinolysis TEG clot lysis time.

Potential carryover effects of Yunnan Baiyao were investigated by use of Wilcoxon rank sum tests to compare between treatment phase 1 and treatment phase 2. These tests were performed with the observed percentage changes for the placebo and results then were compared to detect a difference between horses receiving treatment in the order placebo before or placebo after Yunnan Baiyao for each of the selected 15 variables in the subset.
tal signs or results of CBCs and no clinical signs of coagulopathy.

**In vitro bioassay**

Mean ± SD clotting time for human and equine plasma combined with 5 mg of Yunnan Baiyao/mL (3 replicates/species) was 25.5 ± 0.35 seconds and 31.3 ± 0.28 seconds, respectively. Mean clotting time for human and equine plasma combined with 5 mg of gelatin/mL was 43.4 ± 1.6 seconds and 40.3 ± 0.35 seconds, respectively, and mean clotting time for human and equine plasma combined with an equal volume of buffer was 46.4 ± 0.07 seconds and 42.9 ± 0.71 seconds, respectively. Yunnan Baiyao decreased clotting times, compared with results for the buffer alone, by approximately 40% for human plasma and 30% for equine plasma. Gelatin, however, decreased clotting times by < 10% for both species, which was compatible with a procoagulant effect for Yunnan Baiyao beyond that of an inert contact surface in the assay mixture.

**Hemostasis testing**

Tests used to detect possible carryover effects revealed that horses receiving the placebo after Yunnan Baiyao had significantly (P = 0.026) greater decreases, compared with the baseline value, at 72 hours for platelet-leukocyte aggregates (5 of 6 horses had decreases; median percentage change, -24%) than did horses receiving the placebo before Yunnan Baiyao (only 2 of 6 horses had decreases; median percentage change, 31). The 14 other variables of the 15-variable subset did not have significant (P ≥ 0.13 for all analyses) results when tested for possible carryover effects.

Furthermore, there were no significant (P ≥ 0.15 for all analyses) differences in the percentage changes between 0 and 72 hours for any of the 15 variables in the paired within-horse comparisons between the placebo and Yunnan Baiyao treatments. Results for the Yunnan Baiyao and placebo treatments were summarized (Table 1). The maximal platelet aggregation response to ADP and collagen were correlated (Spearman rank correlation, 0.83; P = 0.001); however, there was no significant enhancement of aggregation in response to Yunnan Baiyao treatment (Figure 1). Similarly, there were significant correlations among several of the flow-cytometric activation variables, including between basal and stimulated PMP (P = 0.006) and between stimulated expression of P selection and PMP (P = 0.013). However, there was no significant effect of Yunnan Baiyao treatment for any of the independent flow-cytometric platelet-activation variables (Figure 2) or for results of assays related to platelet adhesion (closure time, vWF:Ag concentration, and ratio of vWF:Ag concentration to vWF activity de-
termined by use of the vWF:RCo). There also were no significant differences between Yunnan Baiyao and placebo treatments for any of the coagulation variables (aPTT, PT, and fibrinogen concentration) or for WB TEG \( \alpha \) angle. The median baseline values for tPA-triggered plasma fibrinolysis assays were calculated for the 12 study horses (Table 2).

<table>
<thead>
<tr>
<th>tPA (U/mL)</th>
<th>R (min)</th>
<th>( \alpha ) Angle (°)</th>
<th>MA (mm)</th>
<th>LY30 (%)</th>
<th>LY60 (%)</th>
<th>CLT (min)</th>
<th>MRTG (mm/min)</th>
<th>TMRTG (min)</th>
<th>MRL (%)</th>
<th>TMRL (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>3.1</td>
<td>46.5</td>
<td>21.7</td>
<td>62.9</td>
<td>73.5</td>
<td>11.8</td>
<td>6.1</td>
<td>3.4</td>
<td>2.3</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>(3–5)</td>
<td>(11–63)</td>
<td>(5–46)</td>
<td>(0–91)</td>
<td>(0–95)</td>
<td>(5–48)</td>
<td>(0–15)</td>
<td>(0–6)</td>
<td>(0–5)</td>
<td>(9–25)</td>
</tr>
<tr>
<td>500</td>
<td>3.3</td>
<td>37.8</td>
<td>13.5</td>
<td>81.9</td>
<td>88.4</td>
<td>6.0</td>
<td>2.6</td>
<td>1.2</td>
<td>2.8</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Values reported represent results for 2 replicates.

\( \alpha \) Angle = Rate of clot formation. CLT = Clot lysis time. LY30 = Lysis 30 minutes after MA. LY60 = Lysis 60 minutes after MA. MA = Maximal amplitude. MRL = Maximal rate of clot lysis. MRTG = Maximal rate of thrombus generation. R = Reaction time (ie, time to initial clot formation). TG = Rate of thrombus generation. TMRL = Time to maximal rate of lysis. TMRTG = Time to maximal rate of thrombus generation.

Table 2—Median (range) values for TEG variables for plasma fibrinolysis assays of samples obtained before (time 0) oral administration of Yunnan Baiyao and a placebo at 12-hour intervals for 3 days to 12 healthy horses in a crossover study.

In a previous study, there was a reduction in template bleeding time in anesthetized ponies orally premedicated with Yunnan Baiyao, although no effect on activated clotting time was detected. The dose administered in that study (13 to 16 mg/kg) was comparable to the dose administered in the present study. Template bleeding time is an in vivo measurement of primary hemostasis following small-vessel injury, which generally is induced as a small incision distolateral to the accessory carpal bone. The lack of an in vivo bleeding test (such as template bleeding time) was a limitation of the present study. Because the horses were awake, unsedated, and subjected to serial collection of blood samples, and because there is an extremely wide reference range (2.5 to 14 minutes) for a standardized template bleeding time in horses, we opted to use in vitro tests exclusively to assess specific variables of platelet adhesion and aggregate formation in addition to tests of coagulation and fibrinolysis. A possible explanation for the lack of effects in the study reported here was that Yunnan Baiyao acts at endothelial and extravascular cell membrane surfaces at the site of injury, which are conditions present for a template bleeding time test but not for ex vivo assays.

The nanoscale structure of Yunnan Baiyao has been investigated by use of atomic force microscopy, which revealed high concentrations of uniform nanofibers in solution. Authors of that study hypothesized that microscopic nanofibers contribute to the hemostatic effects of Yunnan Baiyao by activating platelets to express a procoagulant surface or by physically adhering to and sealing the site of injury. This theory supports the topical use of Yunnan Baiyao to promote hemostasis at the site of injury or surgical incision and has been corroborated by a report of successful hemostasis via topical use of Yunnan Baiyao for 4 adolescent patients with uncontrolled hemorrhage secondary to advanced cancer. However, conclusions in that report are weakened by a lack of control subjects and concurrent use of conventional hemostatic agents in all patients.

Similar to findings for the present study, prophylactic treatment with Yunnan Baiyao had no benefi-
cific effect on laboratory variables and resulted in no difference in blood loss measured in bronchoalveolar lavage fluid for horses with exercise-induced pulmonary hemorrhage. In that study, horses received 4 g of Yunnan Baiyao orally (approx 50% of the dosage used in the present study) twice daily for 5 days prior to an exercise challenge. It is possible that the lack of Yunnan Baiyao effects in that study were attributable to a low suboptimal dosage or differences in the pulmonary vascular bed compared with those for a template bleeding time wound. Alternatively, pulmonary hemorrhage may not be a manifestation of a hemostatic defect in horses with exercise-induced pulmonary hemorrhage.

The hemostatic effects of Yunnan Baiyao in humans are evident as a reduction in intraoperative blood loss in patients receiving orally administered Yunnan Baiyao as surgical prophylaxis for orthognathic, vertebral, and prostatic procedures. In study, authors reported a reduction in intraoperative blood loss of 21% for patients undergoing bilateral bimaxillary orthognathic surgery. In those prospective, randomized, double-blind, placebo-controlled studies, patients received 0.5 g of Yunnan Baiyao orally 3 or 4 times/d for 3 to 5 days prior to surgery. This dosing schedule is consistent with the recommended labeled dose for oral administration to humans (0.25 to 0.5 g, PO, up to 4 times/d). Yunnan Baiyao dosages for animals are empirical and highly variable. Extrapolation from the recommended human dose results in dosages for horses of approximately 5 g administered orally up to 4 times/d. To emulate dosages typically administered in equine practice, we chose a dose of 15 mg/kg administered orally every 12 hours, which equated to approximately 8 g twice daily in an adult horse. To our knowledge, there are no available pharmacokinetic data for Yunnan Baiyao in horses or humans, and it is possible that the chosen dose did not result in equine plasma concentrations sufficient to induce hemostatic effects reported in human patients.

An additional limitation of the present study was that quantitative analyses of the Yunnan Baiyao preparation to characterize ginseng saponin components was not performed. However, the apparent procoagulant action of the Yunnan Baiyao preparation was confirmed with an in vitro bioassay configured with a snake venom and phospholipid reagent that initiated formation of the prothrombinase complex in plasma through direct activation of factor X. In that assay, the rate of fibrin formation with an in vitro bioassay configured with a snake venom and phospholipid reagent that initiated formation of the prothrombinase complex in plasma through direct activation of factor X. In that assay, the rate of fibrin formation was 2-fold higher in plasma from horses treated with Yunnan Baiyao compared with plasma from control horses. These results suggested that additional pharmacodynamic studies to evaluate hemostatic effects should include in vivo tests and that clinical treatment trials should include higher doses as well as quantitative measures of blood loss. Investigations of the effects of topically applied Yunnan Baiyao by use of in vivo vascular and tissue injury may also provide mechanistic insights to provide a rationale for clinical use of this herbal compound.

Acknowledgments

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The authors thank Dr. Susan Fubini, Melissa Fenn, Brett Robinson, Singen Elliot, Kristina Foyo, Scott Baxendell, and Ian Barrie for technical assistance.

Footnotes

b. Cornell University Equine Park, Ithaca, NY.
d. QuickCalcs, GraphPad Software Inc, La Jolla, Calif.
e. Vacutainer K2 EDTA, BD, Franklin Lakes, NJ.
f. Vacutainer Heparin, BD, Franklin Lakes, NJ.
g. Clinical Pathology Laboratory, Animal Health Diagnostic Center, Cornell University, Ithaca, NY.
h. PFA-100 System, Siemens Diagnostics, Tarrytown, NY.
i. Col/ADP test cartridges, Siemens Diagnostics, Tarrytown, NY.