Hydroxyethyl starch products are artificial non-protein colloids. They are used in veterinary medicine for intravascular volume expansion. They consist of polymers of natural amylopectin, which are chemically modified by hydroxyethylation at the glucose subunit carbon atoms C2, C3, or C6. Hydroxyethyl starch can be classified according to mean Mw, Ms, concentrations, ratio of the C2:C6 hydroxyethylation, the origin of raw material, and the solvent.1,2 These structural aspects modulate the effect on blood coagulation.3 The greater the Mw, the higher the C2:C6 ratio, and especially the higher the Ms, the more an HES product will affect coagulation, and this may lead to an increased risk for hemorrhage.3–8 Hydroxyethyl starch molecules have a specific antiplatelet effect. Among other effects, they can attenuate the availability of the GPIIb/IIIa receptor on the platelet surface, which alters platelet aggregation and clot formation.9

**Objective**—To evaluate the effect of 6% hydroxyethyl starch (HES) solution, with a molecular weight of 130 kDa and a degree of substitution of 0.42, on canine platelet function in vitro.

**Samples**—Blood samples from 31 healthy adult dogs.

**Procedures**—Citrated blood was diluted with saline (0.9% NaCl) solution or HES 130/0.42 in ratios of 1:9 (ie, 1 part saline solution or HES 130/0.42 and 9 parts blood) and 1:3. Platelet plug formation time (closure time [Ct]) was measured with a platelet function analyzer and cartridges coated with collagen and ADP.

**Results**—Median baseline Ct with citrated blood was 84.0 seconds (interquartile range, 74.5 to 99.5 seconds). Results obtained with 1:9 dilutions with saline solution and HES 130/0.42 were not significantly different from baseline results. The 1:3 dilutions with saline solution and HES 130/0.42 resulted in median Cts of 96.0 seconds (interquartile range, 85.5 to 110.8 seconds) and 112.0 seconds (92.0 to 126.0 seconds), respectively. Results obtained with both 1:3 dilutions were significantly different from baseline results. The Ct obtained with the HES dilution was also significantly different from that of the 1:3 dilution with saline solution.

**Conclusions and Clinical Relevance**—Saline solution and HES 130/0.42 in a 1:3 dilution affected canine platelet function by prolonging Cts. The HES 130/0.42 had a significantly greater effect on canine platelets than did saline solution. (Am J Vet Res 2012;73:1908–1912)
A citrated blood sample (800 to 1,000 µL) is aspirated of the vessel wall damage are simulated in this instrument. The primary hemostasis and therefore platelet adhesion, aggregation, and primary platelet plug formation. The PFA has high sensitivity in the assessment of human and canine platelet function disorders and is a less expensive, easier, and faster alternative to platelet aggregation. The cartridge coated with COL-ADP yields more reproducible results in determination of Ct in dogs, compared with a cartridge coated with collagen and epinephrine, because epinephrine is not an effective agonist to platelet activation in dogs. When a COL-ADP cartridge is used, the clinical sensitivity and specificity to identify canine primary hemostasis disorders are 95.7% and 100%, respectively. Anemia and thrombocytopenia, as well as drugs that affect primary hemostasis like acetylsalicylic acid, nonsteroidal anti-inflammatory drugs, and propofol, result in increased Cts. The manufacturer of the PFA guarantees the accuracy of the results when Hct is ≥ 35% and the platelet count is ≥ 150,000 platelets/µL. The objective of the study reported here was to investigate the effect of HES 130/0.42 on in vitro platelet function in canine blood by use of the PFA as compared with saline (0.9% NaCl) solution, in 2 dilutions. We hypothesized that HES 130/0.42 would have a more pronounced effect on in vitro platelet function, compared with saline solution.

Materials and Methods

Animals—The study protocol was approved by the Department of Animal Welfare of the German federal government. Thirty-one healthy adult dogs owned by hospital staff and by consenting clients of the Clinic of Small Animals of the Ludwig Maximilian University were studied. The 31 dogs ranged in age from 1 to 11 years (mean ± SD, 6.23 ± 2.88 years). Mean ± SD weight was 26.55 ± 10.46 kg. Eighteen dogs were females (8 sexually intact; 10 spayed) and 13 were males (4 sexually intact; 9 castrated); there were 12 mixed-breed dogs, 4 Flat-Coated Retrievers, 3 Golden Retrievers, 2 Labrador Retrievers, 2 Beagles, 2 Australian Cattle Dogs, 1 Vizsla, 1 Boxer, 1 Leonberger, 1 Basset Hound, 1 Dalmatian, and 1 Collie. Dogs were considered to be healthy on the basis of history, physical examination, and laboratory findings (CBC, serum biochemical analyses, and urinalysis). Exclusion criteria included history of nonphysiologic bleeding, any chronic or recent illness, and administration of medication known to alter platelet function during the 4 weeks prior to the study. Food was withheld from all dogs for at least 12 hours prior to blood sample collection. Dogs were only included if they had an Hct of ≥ 35%, a platelet count of ≥ 150,000 platelets/µL, a leukogram within reference range, and no pathological findings in serum creatinine concentration, urine dip stick tests, and urine specific gravity.

Blood samples—For determination of platelet function, the PFA was used. The PFA system has been described in detail elsewhere. Briefly, the conditions of vessel wall damage are simulated in this instrument. A citrated blood sample (800 to 1,000 µL) is aspirated under constant vacuum from a reservoir through a capillary microscopic aperture cut into a membrane, which is coated with collagen and epinephrine or ADP. These biochemical stimuli and the high shear flow result in platelet attachment, activation, and aggregation and finally in a stable platelet plug. The time in seconds needed for occluding the aperture by plug formation is called Ct and is indicative of platelet function. Fifteen milliliters of venous blood were obtained with minimal vein trauma from each dog via cephalic, lateral saphenous, or jugular venipuncture (20-gauge needle). After the first drops of blood were discarded, the samples were distributed under continuous blood flow directly into 3.8-mL tubes containing 3.8% buffered sodium citrate solution (1 part anticoagulant to 9 parts blood). In addition, a tube containing K-EDTA for the CBC and a plain tube for serum biochemical analysis were used. According to the manufacturer's recommendations, the citrated blood samples for platelet function analysis were incubated for 30 minutes (stabilization) at room temperature (approx 24°C) prior to analysis. All measurements were completed within 4 hours after blood sample collection. Closure time was measured in duplicate by use of a PFA and COL-ADP. The cartridges were stored at 4°C and warmed to room temperature for 15 minutes before use. Baseline Ct was measured by use of undiluted blood samples (800 µL). Citrated blood was divided into aliquots and diluted with saline solution or HES 130/0.42 at a ratio of 1:9 (1 part saline solution or HES and 9 parts blood; 80 µL of saline solution or HES and 720 µL of blood) and 1:3 (1 part saline solution or HES and 3 parts whole blood; 200 µL of saline solution or HES and 600 µL of blood). After incubation at room temperature for 10 minutes, Cts were measured. Samples with error indication were discarded and repeated.

Statistical analysis—Because repeated duplicated measurements were available, an ordinary least squares linear regression analysis with mixed effects was conducted. That model included a random intercept to handle the unobserved heterogeneity. The random intercept is assumed to be normally distributed. Normality of the residuals was ensured by logarithmic transformation of the dependent variable (Ct). For an identified significant effect, all pairwise multiple comparisons were performed via the Bonferroni adjustment. A P value < 0.05 was considered to be significant. Statistical analysis was performed with software. Data are expressed as median and interquartile range values.

Results

Median Hct was 46% (43% to 50%), and the platelet count was 261,000 platelets/µL (199,000 to 280,000 platelets/µL). Median Ct (Figure 1) for undiluted blood was 84.0 seconds (74.5 to 99.9 seconds). Median Cts for the 1:9 dilution with saline solution and HES 130/0.42 were 84.5 seconds (75.0 to 99.8 seconds) and 88.0 seconds (81.0 to 102.8 seconds), respectively. The 1:3 dilution of blood with saline solution and HES 130/0.42 resulted in Cts of 96.0 seconds (85.9 to 110.8 seconds) and 112.0 seconds (92.0 to 126.0 seconds), respectively.

The Cts from the 1:9 dilution of blood with saline solution (P = 1.00) and HES 130/0.42 (P = 0.982) were...
not significantly different from the undiluted blood (baseline). The Cts from the 1:3 dilution of blood with saline solution and HES 130/0.42 were significantly \( (P < 0.001) \) longer, compared with baseline. The Ct resulting from a 1:3 dilution of blood with HES 130/0.42 was significantly \( (P = 0.023) \) longer than the Ct of the 1:3 dilution of blood with saline solution and the 1:9 dilutions of blood with saline solution \( (P < 0.001) \) and HES 130/0.42 \( (P < 0.001) \). The 1:3 dilution of blood with saline solution resulted in a Ct that was significantly \( (P = 0.003) \) longer than the 1:9 dilutions of blood with saline solution and significantly \( (P = 0.031) \) shorter than that of the 1:3 dilution with HES 130/0.42.

**Discussion**

In this study, the in vitro effect of HES 130/0.42 and saline solution in 2 dilutions on canine platelet function was evaluated by use of the PFA. To improve safety and pharmacological properties while maintaining the volume efficacy of previous HES generations, newer third-generation starch-based plasma volume expanders have been developed. Currently, there are 2 third-generation HES solutions on the market: waxy maize–derived HES 130/0.4 and potato-derived HES 130/0.42; these have known physicochemical differences. A study comparing the effect on coagulation of 3 HES products with differing Mw but the same Ms (HES 130/0.42, 500/0.42, and 900/0.42) suggests that the Mw has less effect than Ms regarding coagulation.

The 1:9 and 1:3 dilutions result in doses of 10 and 30 mL/kg for IV fluid substitution, which are common doses of HES during resuscitation of hypovolemic patients and patients in hemorrhagic shock. In situations of refractory shock, even higher amounts of HES are used, in an attempt to restore effective circulating volume. The recommended dose of high-Mw HES is 20 mL/kg/d. The newer low-Mw HES may be used at doses up to 50 mL/kg/d.

In the present study, the 1:3 dilutions with either saline solution or HES 130/0.42 significantly prolonged Cts, whereas the 1:9 dilutions did not. The prolongation of Ct with saline solution can be explained by the effect of dilution. Crystalloids cause alterations because of simple dilution of clotting factors. The Ct of the 1:3 dilution with HES 130/0.42 was significantly prolonged, compared with the 1:3 dilution with saline solution. This indicates that the HES 130/0.42 in a 1:3 dilution exaggerates the dilutional effect on platelet function and that HES 130/0.42 has a more pronounced effect on platelet function in vitro than saline solution. Because a significant effect of HES 130/0.42 was only detected in a 1:3 dilution, the effect appears to be dose dependent. Hydroxyethyl starch products compromise hemostasis and platelet function via either nonspecific dilutional effects, specific effects, or both. They are known to induce acquired von Willebrand disease, favor fibrinolysis through the incorporation of HES molecules in the clot, and alter platelet aggregation by modification of the platelet membrane and inhibition of conformational activation of the GPIIb/IIIa complex. Another recent study revealed that HES macromolecules are able to bind to platelets and thus impair the access of ligands to the platelet fibrinogen receptor (GPIIb/IIIa). These mechanisms provide a plausible explanation for the observed prolongation of Ct.

In the present study, the Cts for citrated blood of 31 healthy dogs ranged from 57 to 138 seconds with a mean \( \pm SD \) of 87.7 \( \pm 18.9 \) seconds and a median of 84.0 seconds (interquartile range, 74.5 to 99.5 seconds). Measurements in other studies using PFA-100 and COL-ADP resulted in mean \( \pm SD \) Cts of 67 \( \pm 7.8 \) seconds with a range of 52 to 86 seconds \( (n = 29) \) in one study and 57.6 \( \pm 5.9 \) seconds with a range of 48 to 77 seconds \( (n = 58) \) in another. A median Ct of 73 seconds with a 2.5% to 97.5% quantile range of 53 to 98 seconds \( (n = 136) \) is also reported. These values are not exactly identical to the results of the present study. It is known that many factors like age, sex, nutrition, differences of the test cartridge, different citrate concentrations in the sample tubes, regional variations, and daily variations may have an effect on Cts in humans, which is probably also the case in dogs. It is also known that the PFA should considered a semiquantitative method. Although the manufacturer of the PFA only validated Cts to be accurate for a platelet count of \( > 150,000 \) platelets/µL and Hct of \( > 35\% \), a veterinary study found the PFA to be accurate even for Hct \( \geq 29\% \) and for platelet count \( \geq 100,000 \) platelets/µL. In the present study, Hct and platelet count in 1:3 dilution had been calculated. The median Hct would be \( 35\% \) with a range of 29% to 47%, and the range of platelet counts would be \(~113,250\) to \(~271,500\) platelets/µL. Therefore, Cts measured with these 1:3 diluted samples can be considered an accurate evaluation of platelet function. In line with our results, various in vitro studies have found that HES 130/0.4 has a greater effect on platelet function and hemostasis than crystalloids. These findings are similar to the find-
nings of Liu et al, 17 who studied the influence of HES 130/0.4, HES 200/0.5, and saline solution on human platelet function in vitro in different dilutions by using a PFA, platelet aggregometry, and erythrocyte sedimentation rate. They found that both HES solutions significantly prolonged Cts in a dose-dependent manner and to a significantly greater extent than saline solution. An in vivo study41 compared the influence of HES 130/0.4, HES 200/0.5, and lactated Ringer’s solution on platelet function and hemodynamics in 40 adult humans. That study found a significant prolongation of Cts after an infusion of only 10 mL/kg with both HES solutions, whereas lactated Ringer’s solution in the same dose did not result in prolongation of Cts. In contrast, another study12 found no significant effect on Ct after IV infusion (10 mL/kg) of HES 130/0.38 to 0.45 in healthy human patients. This discrepancy may be due to different patient populations. In the present in vitro study, there was also no effect with a simulated dose of 10 mL/kg.

In a European prospective multicenter observational postauthorization safety study in 316 children receiving HES 130/0.42 (mean ± SD, 11 ± 4.8 mL/kg) periperatively, no serious adverse drug reactions directly related to HES were reported. The probability of serious adverse drug reactions like bleeding was < 1%. 42

In veterinary medicine, only a few reports regarding the effect of HES on canine platelet function and coagulation are published to date. In a recent study, 13 the in vitro effects of HES 130/0.4, hetastarch, and saline solution in healthy dogs were compared by use of thrombelastography. This revealed that HES 130/0.4 causes less effects on coagulation than hetastarch in a high dilution, and it was concluded that a dilutional coagulopathy does not seem to be the only cause of the HES 130/0.4–induced effect on coagulation. In another study, 13 the in vitro effect of HES 670/0.75 and HES 600/0.7 on platelet function in dogs measuring Cts with a PFA in 2 dilutions was compared. Those investigators found a significant effect only in the highest dilution (1:3), and the Ct at 1:3 dilution for HES 670/0.75 was significantly higher than for dilution with saline solution. Further canine in vitro studies with different HES preparations are necessary to determine whether the current findings in human medicine, which indicate that HES products with lower Mw and Ms have a significantly reduced effect on coagulation compared with older products, are also true for dogs. An in vivo study14 revealed that a 20 mL/kg dose of HES 600/0.75/ kg prolongs canine Ct, as measured by PFA, for up to 5 hours after injection, although Ct returned to baseline within 24 hours. No clinical signs of bleeding, such as petechiae or ecchymoses, were observed during the experimental period. In addition, no significant change in platelet aggregation (measured with an electronic aggregometer) and no clinical bleeding during surgery were observed in a recent study9 that compared the effect of a 10 mL/kg dose of HES 600/0.75/kg or the same volume of lactated Ringer’s solution on hemostatic variables and bleeding in dogs anesthetized for orthopedic surgery. Because of these results, the more important question is how clinically relevant these in vitro impairments of platelet function really are. Although there was a significant difference between the Cts in the 1:9 and 1:3 dilutions and especially between the 1:3 dilution with saline solution and HES 130/0.42, there was a substantial overlap in measured values in the present study. Despite the reported high clinical sensitivity and specificity in the identification of primary hemostatic disorders in dogs, 16 it can be concluded that the clinical relevance of these in vitro findings is not obvious.

The present study had the limitations of in vitro models. Compensatory mechanisms like buffering, pH control, and electrolyte environment are not the same as in vivo. Also, the metabolic degradation of HES molecules by plasma α-amylose and the contribution of endothelial and vascular factors are restricted. Even if the PFA simulates the conditions of a damaged vessel under high shear flow, it can only mimic the in vivo conditions. Consequently, the results could not be directly translated to the clinical setting. It can be concluded that HES 130/0.42 in a high concentration (30 mL/kg) prolonged Ct in vitro compared to saline solution in the same concentration. These findings indicate that, despite the lower Mw and the lower Ms, HES 130/0.42 has a more pronounced effect regarding in vitro platelet function than saline solution. Therefore, possible adverse effects, in particular impairment of hemostasis, should be kept in mind, especially in patients with already compromised hemostasis.

References


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e. 3.8 mL S-Monovette for PFA-100, SARTEDT, Nürnberg, Germany.

f. SPSS Advanced Statistics, version 21.0, IBM Germany GmbH, Ehningen, Germany.


