In vitro effect of hydroxyethyl starch on coagulation in dogs as assessed by dynamic viscoelastic coagulometry

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OBJECTIVE
To evaluate the effect of 6% hydroxyethyl starch (HES) 670/0.75 and 6% HES 130/0.4 dilution of canine whole blood on coagulation using dynamic viscoelastic coagulometry (DVC).

ANIMALS
56 healthy adult dogs.

PROCEDURES
2 blood samples were obtained from each dog and randomized to 1 of 7 groups—undiluted or 2 dilutions (1:3 or 1:10) of 3 different fluids: saline (0.9% NaCl) solution, 6% HES 670/0.75, or 6% HES 130/0.4. Dilutions were calculated to simulate approximately a 10- or 30-mL/kg body weight IV bolus of each fluid. DVC was performed on each sample. Coagulation parameters compared between groups included clot rate (CR), platelet function (PF), and activated clotting time.

RESULTS
Dilution with saline solution did not significantly affect coagulation, while dilution with HES 670/0.75 and HES 130/0.4 caused a dose-dependent significant decrease in CR (1:3 HES 670/0.75, P = 0.007; 1:10 HES 670/0.75, P = 0.002; 1:3 HES130/0.4, P < 0.0001; and 1:10 HES 130/0.4, P < 0.0001; 1:10 HES 670/0.75, P < 0.0001; 1:3 HES130/0.4, P < 0.0001; and 1:10 HES 130/0.4, P = 0.0015).

CLINICAL RELEVANCE
Dilution of canine blood with HES 670/0.75 and HES 130/0.4, at clinically relevant doses (10 and 30 mL/kg), led to significant hypocoagulability beyond dilutional effect. This was, in part, due to impaired PF, which was significantly greater with HES 670/0.75. Further research using DVC to assess the effects of HES on coagulation in dogs, ideally with clinical conditions warranting HES administration, is needed.
IV bolus, HES effects on coagulation can last > 24 hours with HES 600 to 675/0.75 and generally last less than 24 hours with HES 130/0.4. 5–7

Dynamic whole blood viscoelastic tests of coagulation have been used in dogs to assess coagulation in health and disease. Commercially available devices include rotational thromboelastometry, thromboelastography, and dynamic viscoelastic coagulometry (DVC; Sonoclot coagulation and platelet function analyzer; Sienco Inc). All of these devices provide information regarding clot dynamics—rate of clot formation, strength of the clot, and clot lysis. To date, all published dynamic whole blood viscoelastic coagulation studies 1–3,11,12,22 have specifically assessed PF using thromboelastography or rotational thromboelastometry.

Dynamic viscoelastic coagulometry uses a disposable cuvette and hollow pin that oscillates vertically within the blood sample, detecting changes to viscoelasticity as the clot forms. It provides a signature tracing, which is a graphical representation of clot formation over time, and coagulation parameters including clot rate (CR), PF, and activated clotting time (ACT). Clot rate represents the initial fibrin polymerization and clot development and is related to fibrinogen concentration, quality of thrombin, and PF. 16,17 Platelet function is provided as a value between 0 and 5, which is determined based on the timing and quality of clot retraction. 16,17 In humans, PF significantly correlates to platelet number and function as assessed by aggregometry. 18–20 In healthy dogs, PF significantly correlates to platelet count, but there are no published data comparing it to other tests of PF. 21 Activated clotting time is the time to the beginning of fibrin formation and is related to soluble coagulation factors and endogenous anticoagulants. 17

It is unknown if DVC can be used to assess the effects of HES on coagulation in dogs. This study was designed to test the hypothesis that the addition of HES 670/0.75 and HES 130/0.4 to canine whole blood would lead to a dose-dependent relative hypoagglutinability that could be demonstrated with DVC.

Materials and Methods

Animals

This study was approved by the Institutional Animal Care and Use Committee at Oklahoma State University. Healthy dogs owned by students, faculty, and staff of the Veterinary Medical Hospital at Oklahoma State University were recruited for the study. Signed and informed pet-owner consent was obtained before study inclusion. Inclusion criteria included being considered healthy based on provided medical history, physical examination, and evaluation of a CBC, serum biochemistry analysis, prothrombin time (PT), and partial thromboplastin time (PTT). Exclusion criterion included administration of a medication known to affect coagulation, including, but not limited to, nonsteroidal anti-inflammatories, corticosteroids, aspirin, clopidogrel, warfarin, or heparin during the month prior to enrollment.

Blood collection

Blood was collected from each dog via a traumatic stick jugular venipuncture using a 20-gauge, 1-inch needle and 6-mL syringe. Two 4-mL blood samples were obtained with at least 1 h between collections. The second sample was drawn from the contralateral jugular vein. A CBC (AU680 Clinical Chemistry Analyzer; Beckman Coulter; Antech Diagnostics), serum biochemistry analysis (AU680 Clinical Chemistry Analyzer), PT (KC4 Delta; Tcoag; Antech Diagnostics), and PTT (KC4 Delta) were performed on the first sample obtained. Platelet estimates were automated; if low, a manual platelet count was performed by a laboratory technician and reviewed by a clinical pathologist. Each sample was placed into 2 commercially available blood tubes (BD Vacutainer citrate tubes) containing 3.8% trisodium citrate (1 part citrate: 9 parts blood) and stored upright, without agitation, at room temperature until DVC analysis.

Study design

This study was a partially balanced, incomplete random block design with each blood sample being randomly assigned to 1 of 7 groups: (1) no dilution; (2) 1:3 dilution with saline (0.9% NaCl) solution (9% sodium chloride injection, Baxter Healthcare Corp); (3) 1:10 dilution with saline solution; (4) 1:3 dilution with 6% HES 670/0.75 (Hespan; Hospira Inc) in saline solution; (5) 1:10 dilution with 6% HES 670/0.75 in saline solution; (6) 1:3 dilution with 6% HES 130/0.4 (Vetstarch; Zoetis) in saline solution; and (7) 1:10 dilution with 6% HES 130/0.4 in saline solution. The dilutions were equivalent to a 10- or 30-mL/kg IV bolus of each fluid, which was similar to dilutions used in previous in vitro studies 1–11,12,22 in dogs.

Dynamic viscoelastic coagulometry

A single dual-chamber dynamic viscoelastic coagulometer (Sonoclot coagulation and platelet function analyzer; Sienco Inc) was used for all DVC analyses performed by one operator. Routine maintenance and quality control procedures included daily calibration with a reference viscosity standard, monthly evaluation of control plasma samples, and visual inspections of signature tracings. Glass bead–activated cuvettes (gbACT+; Sienco Inc) were used for all assays. For control samples, 330 μL of citrated whole blood was added to 30 μL of 0.2 M CaCl2 (Fisher Chemical) in a cuvette warmed to 37°C. For 1:3 dilution samples, 248 μL of citrated whole blood and 90 μL of diluent (saline solution, HES 670/0.75, or HES 130/0.4) were added to 22 μL of 0.2 M CaCl2 in a cuvette warmed to 37°C. For 1:10 dilution samples, 300 μL of citrated whole blood and 33 μL of diluent (saline solution, HES 670/0.75, or HES 130/0.4) were added to 27 μL of 0.2 M CaCl2 in a cuvette warmed to 37°C. All DVC analyses lasted for 30 minutes or until the CR, PF, and ACT were reported.
Statistical analysis

Statistical analysis was performed with commercial software (SAS 9.4; SAS Institute, Cary, NC). Descriptive statistics were presented as median (range) or mean (SE). Mean DVC coagulation parameters (ACT, CR, and PF) were compared between groups using ANOVA with protected pairwise least significant difference. Statistical significance was set at $P < 0.05$.

Results

Animals and routine laboratory work

Fifty-six dogs with a median age of 4 years (range: 1 to 8 years) and median body weight of 20.4 kg (range, 4.8 to 63.8 kg) were included in the study. There were 32 females (5 sexually intact and 27 spayed) and 24 males (1 sexually intact and 23 castrated). Mixed breed dogs ($n = 18$) and 23 different purebred dogs were represented ($38$).

All venipuncture was performed appropriately for adequate processing of samples and adequate reporting. All assays were also performed without encountering any concerns. Prothrombin time, PTT, and Hct were within reference intervals for all dogs. Platelet counts were within reference intervals or estimated to be adequate with platelet clumping in all dogs. Mild abnormalities on CBC or serum biochemistry analysis were found in 5 dogs, including hyperglobulinemia in 2 dogs (4.3 and 4.3 g/dL; reference range, 1.6 to 3.6 g/dL), increased serum alanine aminotransferase activity in 1 dog (164 U/L; reference range, 12 to 118 U/L), and eosinophilia in 2 dogs (1,204 and 1,515 cells/$\mu$L; reference range, 0 to 1,200 cells/$\mu$L). These abnormalities were considered clinically insignificant, and the dogs were included in the study.

Dynamic viscoelastic coagulometry

Coagulation results were tabulated (Table 1). In summary, both HES 670/0.75 and HES 130/0.4 at 1:10 and 1:3 dilutions resulted in a significant decrease in CR when compared with no dilution or dilution with an equal volume of saline solution (Figure 1). Saline solution at either dilution did not significantly affect CR when compared with no dilution. Both HES 670/0.75 and HES 130/0.4 at both dilutions caused a significant decrease in PF as compared with no dilution and dilution with equal volumes of saline solution (Figure 2).

![Figure 1](image-url)
either dilution did not significantly affect PF when compared with no dilution. Only saline solution at the 1:3 dilution and HES 130/0.4 at the 1:3 dilution caused a significant decrease in ACT compared with no dilution (Figure 3). These were not significantly different from ACT with other dilutions of saline, HES 670/0.75, or HES 130/0.4.

**Discussion**

To the authors’ knowledge, this was the first study to investigate the effects of HES on canine whole blood coagulation using DVC. In this study, dilution of canine whole blood with either HES dilutions of HES, different coagulation devices or PF analyzers, different activators, and other preanalytical and analytical variabilities.

This study demonstrated significantly greater impairment of PF with HES 670/0.75 as compared with equal volumes of saline, HES 670/0.75, or HES 130/0.4. This contradicts a previous in vitro study that failed to show a significant difference in PF between HES 670/0.75 and HES 130/0.4 in canine blood. Overall, there is reason to believe that HES solutions with higher molecular weights and degrees of substitution do lead to relatively more impairment of PF, as findings of impaired PF have been more consistent with HES 670/0.75, compared with HES 130/0.4.
shown that higher molecular weight, degree of substitution, and C2:C6 ratio lead to slower metabolism and thus a longer duration of effect.\textsuperscript{15,25,26} In addition, one canine study\textsuperscript{22} found significantly greater impairment of PF with HES 200/0.5, when compared with an equal volume of HES 130/0.4. Findings of relatively greater impairment of PF with a higher molecular weight and degree of substitution HES solution in the present study are consistent with previously reported data in humans.\textsuperscript{27,28}

Proposed reasons that HES might cause impairment of PF include inhibition or decreased expression of glycoprotein (GP) IIb/IIIa, also known as integrin αIIbβ3, and decreased expression of cell surface serine phospholipids, among others.\textsuperscript{29,30} Glycoprotein IIb/IIIa plays a significant role in platelet adhesion and aggregation. Once a platelet is activated, GP IIb/IIIa undergoes a conformational change to allow for an increased affinity of the receptor for binding of fibrinogen. With fibrinogen binding, adjacent platelets can undergo a facilitated interaction and GP IIb/IIIa undergoes further conformational change to allow for an acceleration of platelet aggregation.\textsuperscript{31} Dynamic viscoelastic coagulometry is a sensitive indicator of impaired PF secondary to GP IIb/IIIa inhibition, caused by tirofiban.\textsuperscript{19} Tirofiban also affects closure time, as measured by the Platelet Function Analyzer-100, a device commonly used in the published literature of the effects of HES in dogs.\textsuperscript{18} The findings of the present study suggested that DVC is a sensitive indicator of impaired PF due to HES in dogs. As such, advantages of DVC might include a single test that can provide a global view of whole blood coagulation along with easy-to-obtain information regarding the contribution of PF. Further research, including the in vivo effects of colloids on whole blood coagulation as assessed by DVC, is needed.

In addition to PF, CR was significantly prolonged with both dilutions of both HES solutions in the present study. This effect was not seen with equal volumes of saline solution. The significantly lower CR was likely, at least partly, due to the noted impaired PF. Different from PF, a dose-dependent effect was not seen with CR, which might suggest that a factor other than PF is also affecting CR. Clot rate is also affected by thrombin and fibrinogen concentrations. In vitro HES 130/0.4 has been shown to decrease factor VIII activity and serine phospholipid expression, thus decreasing thrombin generation.\textsuperscript{29} Significant effects of thrombin or fibrinogen concentrations are not supported by relative lack of significant changes seen to ACT. As a result of design, in this study, investigation of changes to specific coagulation factor activity, phospholipid expression, thrombin generation, fibrinogen concentration, or other potential variables was not possible.

Activated clotting time is not significantly affected by platelet count or PF. In the present study, the changes to ACT were small and inconsistent between fluid types and dilutions. In general, HES dilution did not significantly affect ACT as compared with samples with no dilution or dilution with saline solution. This is consistent with other findings in this study that suggest that impaired PF, and not simple dilutional effects, explain the relative hypocoagulability demonstrated with HES. As evaluated by DVC, collectively these findings suggest that HES has only relatively small effects on soluble coagulation factors and endogenous anticoagulants in canine blood.

Our study had multiple limitations, some of which are inherent to in vitro studies. These include the exclusion of endothelial cell, blood flow dynamics, HES pharmacokinetics, and many other complex physiological factors that are not recreated with DVC. A second limitation included the use of healthy dogs. In clinical practice, HES is expected to be commonly used in unhealthy dogs requiring volume expansion or oncotic support. Using healthy dogs excludes many possible pathologic factors that might affect coagulation, such as protein-losing disease, systemic inflammation, cancer, and immune-mediated disease, among others. A third limitation was that our study was designed to investigate a fluid bolus scenario and HES is also administered over longer periods of time clinically. Immediate platelet inhibition has been demonstrated in humans after a bolus of HES, suggesting that even short-term exposure to HES in vitro could mimic those in vivo. It is unknown how these effects would be altered by longer duration lower concentration exposure, as could be seen with a constant rate infusion of HES.\textsuperscript{32} A final limitation included the fact that an additional PF analyzer was not used. Although there is evidence that DVC demonstrates PF in humans,\textsuperscript{18,20} it has not been validated for PF testing in dogs.

In conclusion, dilution of canine blood with HES 670/0.75 and HES 130/0.4 at clinically relevant doses (10 and 30 mL/kg) led to significant hypocoagulability beyond simple dilutional effects as evaluated by DVC. This was at least partly due to impaired PF, which was significantly greater with HES 670/0.75 as compared with HES 130/0.4. Further research using DVC to assess the effects of HES on coagulation in dogs, ideally with clinical conditions warranting HES administration, is needed.

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References

1. Falco S, Bruno B, Maurella C, et al. In vitro evaluation of canine hemostasis following dilution with hydroxyethyl starch (130/0.4) via thromboelastometry. J Vet Emerg Crit Care. 2012;22(6):640–645. doi:10.1111/j.1476-4431.2012.00816.x.

2. Morris BR, deLaforcade A, Lee J, Palmisano J, Meola D, Rozanski E. Effects of in vitro hemodilution with crystalloids, colloids, and plasma on canine whole blood coagulation as determined by kaolin-activated thromboelastography. J Vet Emerg Crit Care. 2016;26(1):58–63. doi:10.1111/vec.12345.
Sarin SK. Sonoclot signature analysis in patients with liver disease and its correlation with conventional coagulation studies. Adv Hematol. 2013;2013:237351. doi:10.1155/2013/237351.

Tucci MA, Ganter MT, Hamiel CR, Klaghofer R, Zollinger A, Hofer CK. Platelet function monitoring with the Sonoclot analyzer after in vitro tirofiban and heparin administration. J Thorac Cardiovasc Surg. 2006;131(6):1314–1322. doi:10.1016/j.jtcs.2006.01.041.

Miyashita T, Kuro M. Evaluation of platelet function by Sonoclot analysis compared with other hemostatic variables in cardiac surgery. Anesth Analg. 1998;87(6):1228–33. doi:10.1097/00000539-199812000-00002.

Babksi DM, Brainard BM, Krimer PM, Ralph AG, Pittman JR, Koenig A. Sonoclot evaluation of whole blood coagulation in healthy adult dogs. J Vet Emerg Crit Care. 2012;22(6):646–652. doi:10.1111/j.1476-4431.2012.00820.x.

McBride D, Hosgood GL, Mansfield CS, et al. Effect of hydroxyethyl starch 130/0.4 and 200/0.5 solutions on canine platelet function in vitro. Am J Vet Res. 2013;74(8):1133–1137. doi:10.2460/ajvr.74.8.1133.

Krzycz L, Czempik PF. Effect of fluid resuscitation with balanced solutions on platelets: In vitro simulation of 20% volume substitution. Cardiol J. 2018;25(2):254–259. doi:10.5603/CJ.a2017.0054.

McBride D, Hosgood G, Raisis A, Smart L. Platelet closure time in anesthetized greyhounds with hemorrhagic shock treated with hydroxyethyl starch 130/0.4 or 0.9% sodium chloride infusion. J Vet Emerg Crit Care. 2016;26(4):509–515. doi:10.1111/j.1476-4431.2012.00820.x.

Treib J, Haass A, Pindur G, et al. HES 200/0.5 is not HES 200/0.5. Influence of the C2/C6 hydroxyethylstarch ratio of hydroxyethyl starch (HES) on hemorheology, coagulation and elimination kinetics. Thromb Haemost. 1995;74(6):1452–1456.

Treib J, Haass A, Pindur G, Grauter M, Wenzel E, Schmirigk K. All medium stanches are not the same: influence of the degree of hydroxyethyl substitution of hydroxyethyl starch on plasma volume, hemorrhheologic conditions, and coagulation. Transfusion. 1996;36(5):450–455. doi:10.1046/j.1537-2995.1996.36596282590.x.

Entholzer EK, Mielke LL, Calatzis AN, Feih J, Hipp R, Hargasser SR. Coagulation effects of a recently developed hydroxyethyl starch (HES 130/0.4) compared to hydroxyethyl stachers with higher molecular weight. Acta Anaesthesiol Scandin. 2000;44(9):1116–1121. doi:10.1034/j.1399-6576.2000.440914.x.

Liang H, Yang C, Zhang B, et al. Hydroxyethyl starch 200/0.5 decreases circulating tumor cells of colorectal cancer patients and reduces metastatic potential of colon cancer cell line through inhibiting platelets activation. Med Oncol. 2015;32(5):151. doi:10.1007/s12032-015-0601-3.

Jin S, Yu G, Hou R, Shen B, Jiang H. Effect of hemodilution in vitro with hydroxyethyl starch on hemostasis. Med Sci Monit. 2017;23:2189–2197. doi:10.12659/msm.901588.

Chen G, Yan M, Lu QH, Gong M. Effects of two different hydroxyethyl starch solutions (HES200/0.5 vs. HES130/0.4) on the expression of platelet membrane glycoprotein. Acta Anaesthesiol Scand. 2006;50(9):1089–1094. doi:10.1111/j.1399-6576.2006.01136.x.

Fullard JF. The role of the platelet glycoprotein IIb/IIIa in thrombosis and haemostasis. Curr Pharm Des. 2004;10(14):1567–1576. doi:10.2174/138161204384682.

Franz A, Braunlich P, Gamsjager T, Felfering M, Gauthier V, Holowaychuk MK, Kerr CL, Bersenas AM, Wood RD. Effect of synthetic colloid administration on coagulation in healthy dogs and dogs with systemic inflammation. J Vet Intern Med. 2015;29(1):276–285. doi:10.1111/jvim.12492.

Reuteler A, Axiaflammer S, Howard J, Adamik K. Comparison of the effects of a balanced crystalloid-based and a saline-based tetraslarch solution on canine whole blood coagulation and platelet function. J Vet Emerg Crit Care. 2017;27(1):23–34. doi:10.1111.jvac.12556.

Smart L, Jandrey KE, Kass PH, Wierenga JR, Tablin F. The effect of Hetastarch (670/0.75) in vivo on platelet closure time in the dog. J Vet Emerg Crit Care. 2009;19(5):444–449. doi:10.1111/j.1476-4431.2009.00464.x.

Chohan AS, Greene SA, Grubb TL, Keegan RD, Wills TB, Martinez SA. Effects of 6% hetastarch (600/0.75) or lactated Ringer’s solution on hemostatic variables and clinical bleeding in healthy dogs anesthetized for orthopedic surgery. Vet Anaesth Analg. 2011;38(2):94–105. doi:10.1111/j.1476-2995.2010.00589.x.

Helmbold KA, Mellema MS, Hopper K, Epstein S. The effect of hetastarch 670/0.75 administered in vivo as a constant rate infusion on platelet closure time in the dog. J Vet Emerg Crit Care. 2014;24(4):381–387.

Botto A, Bruno B, Maurella C, et al. Thromboelastometric assessment of hemostasis following hydroxyethyl starch (130/0.4) administration as a constant rate infusion in hypovolemic dogs. BMC Vet Res. 2018;14(1):33. doi:10.1186/s12917-018-1157-8.

Griego-Valles M, Burko Y, Prittie JE, Fox PR. An in vitro comparison of the effects of voluven (6% hydroxyethyl starch 130/0.4) and hepan (6% hydroxyethyl starch 670/0.75) on measures of blood coagulation in canine blood. J Vet Emerg Crit Care. 2017;27(1):44–51. doi:10.1111/jvac.12541.

Wierenga JR, Jandrey KE, Haskins SC, Tablin F. In vitro comparison of the effects of two forms of hydroxyethyl starch solutions on platelet function in dogs. Am J Vet Res. 2007;68(6):605–609. doi:10.2460/ajvr.68.6.605.

Treib J, Haass A, Pindur G. Coagulation disorders caused by hydroxyethyl starch. Thromb Haemost. 1997;78(3):974–983.

Koek-Langenecker SA. Effects of hydroxyethyl starch solutions on hemostasis. Anesthesiology. 2005;103(3):654–660. doi:10.1097/00000542-200509000-00031.

Diniz MS, Teixeira-Neto FJ, Goncalves DS, et al. Effects of 6% tetraslarch or lactated Ringer’s solution on blood coagulation in hemorrhaged dogs. J Vet Intern Med. 2018;32(6):1927–1933. doi:10.1111/jvim.15327.

Ganter MT, Hofer CK. Coagulation monitoring: current techniques and clinical use of viscoelastic point-of-care coagulation devices. Anesthes Analg. 2008;106(5):1366–1375. doi:10.1213/ane.0b013e318168b367.

Yamada T, Katori N, Tanaka KA, Takeda J. Impact of Sonoclot hemostasis analysis after cardiopulmonary bypass on postoperative hemorrhage in cardiac surgery. J Anesth. 2007;21(2):148–152. doi:10.1007/s10540-006-0477-7.

Saxena P, Bihari C, Rastogi A, Agarwal S, Anand L, Sarin SK. Sonoclot signature analysis in patients with