Effect of hydroxyethyl starch 130/0.4 and 200/0.5 solutions on canine platelet function in vitro

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Objective—To determine whether dilution of blood samples from healthy dogs with 2 hydroxyethyl starch (HES) solutions, HES 130/0.4 and HES 200/0.5, would result in platelet dysfunction as measured by closure time (Ct) beyond a dilutional effect.

Sample—Citrated blood samples from 10 healthy dogs with a Ct within reference limits (52 to 86 seconds).

Procedures—Blood samples were diluted 1:9 and 1:3 with 6% HES 130/0.4 and 10% HES 200/0.5 solutions and saline (0.9% NaCl) solution. Dilutions at 1:9 and 1:3 mimicked 10 mL/kg and 30 mL/kg doses, respectively, ignoring in vivo redistribution. Closure time was measured with a platelet function analyzer and compared among dilutions.

Results—A dilutional effect on Ct was evident for the 1:3 dilution, compared with the 1:9 dilution, but only HES 200/0.5 increased the Ct beyond the dilutional effect at the 1:3 dilution, to a median Ct of 125 seconds (interquartile range, 117.5 to 139.5 seconds). No effect of HES or dilution on Ct was identified at the 1:9 dilution.

Conclusions and Clinical Relevance—1:3 dilution of blood samples from healthy dogs with HES 200/0.5 but not HES 130/0.4 significantly increased Ct beyond the dilutional effect, suggesting that IV administration of HES 200/0.5 in dogs might cause platelet dysfunction. (*Am J Vet Res* 2013;74:1133–1137)

Hydroxyethyl starch solutions are artificial colloid solutions used in dogs to treat shock by expanding blood volume. The use of HES is controversial because of its association with platelet dysfunction in people[1–5] and dogs,[6–8] and in human medicine, its use has been associated with an increase in intraoperative blood loss and transfusion requirements.[1,9–12] This effect is particularly relevant to solutions with a high molecular weight and molar substitution,[1,9,10] such as the commonly used solution HES 670/0.75, which has a high molecular weight of 670 kDa and a high molar substitution of 0.75.

A platelet function analyzer can be used to assess platelet function in high shear stress conditions.[13] The device measures Ct, which is the time taken in seconds for a small aperture, coated with a platelet agonist, to be closed by platelet plug formation.[13] An increase in

Abbreviations

Ct	Closure time
HES	Hydroxyethyl starch
IQR	Interquartile range

Ct beyond the reference interval of 52 to 86 seconds,[14] through use of cartridges coated with collagen and ADP as a platelet agonist, indicates platelet dysfunction in dogs. Dilution of blood that results in a decrease in Hct or PCV (< 25%[15] to 30%[14,16]) and platelet count (< 100,000 platelets/μL[14]) can also increase Ct.

Two studies[6,7] assessed the effect of HES 670/0.75 on canine platelet function by use of the PFA-100. Canine blood samples diluted at a ratio of 1:3 with HES 670/0.75 solution led to an increase in Ct,[6] and HES 670/0.75 administered IV to healthy dogs at a dose of 20 mL/kg also resulted in an increase in Ct.[6,7] The findings of both studies support the hypothesis that use of HES 670/0.75 can cause platelet dysfunction in dogs, although the effect of dilution alone on Ct in the second study was not specifically addressed. In another study[8] in which HES 670/0.75 was administered to anesthetized dogs at a dose of 10 mL/kg IV, platelet aggregometry revealed no difference in platelet function, compared with platelet function after administration of lactated Ringer's solution.

Because of the complications associated with the use of HES 670/0.75 in people, HES solutions with lower molecular weights and molar substitutions, such as

HES 130/0.4 and HES 200/0.5, were developed to minimize adverse effects, such as platelet dysfunction. Evidence for a difference between these 2 HES solutions, however, is mixed in regard to their effect on platelet function in people. Whereas some studies[2,17,18] have indicated that administration of HES 130/0.4 does not cause platelet dysfunction, others have shown that use of HES 130/0.4 causes platelet dysfunction to the same[4] or lesser[5] degree than that caused by HES 200/0.5. No consensus has been reached on the clinical implication of using an HES solution of lower molecular weight and molar substitution. Although a study[19] has shown some differences in blood loss and transfusion requirements among people treated with HES solutions of different molecular weights and molar substitutions, several meta-analyses have failed to show a significant difference.[1,20,21]

Little research has been conducted to investigate the effect of HES solutions of low molecular weight and molar substitution on platelet function in dogs. One study[22] revealed that dilution of canine blood samples with HES 130/0.42 at a ratio of 1:3 resulted in an increase in Ct beyond a dilutional effect.[22] The product HES 130/0.4, which has a low molecular weight and molar substitution and is commercially available in North America and Australia, has different properties from HES 130/0.42. The product HES 200/0.5 is also available in Australia.

Whether use of HES 130/0.4 causes platelet dysfunction in dogs is unknown, as is whether use of HES 200/0.5 would cause worse platelet dysfunction because of its higher molecular weight and molar substitution than HES 130/0.4. The purpose of the study reported here was to measure Ct in samples of canine whole blood diluted with HES 130/0.4 and HES 200/0.5 in vitro and to compare the results with those of whole blood diluted with saline (0.9% NaCl) solution. We hypothesized that dilution alone would result in a dilution-dependent increase in the Ct and that whole blood samples diluted with HES 200/0.5 would have a longer Ct than those diluted with HES 130/0.4, beyond any dilutional effect.

Materials and Methods

Animals—Ten healthy adult dogs (6 males and 4 females) owned by hospital staff members were included in the study after owner consent was obtained. The median age of the dogs was 4 years (IQR, 2 to 8 years), and the median body weight was 28.2 kg (IQR, 16.2 to 31.2 kg). All dogs were considered healthy on the basis of the absence of the following factors: disease or illness within 1 month prior to study inclusion; history of coagulopathy or disease that may affect hemostasis; treatment with NSAIDs, propofol, synthetic colloids, or antimicrobials within 1 month prior to study inclusion; and abnormal findings during physical examination. Each dog had a PCV > 40%; platelet count > 200 $\times 10^9$ cells/L as measured with a hematologic analyzer[a] and verified by manual count; mean Ct within the reference interval of 52 to 86 seconds,[14] as measured in duplicate with the platelet function analyzer[a] by use of cartridges coated with collagen and ADP[b]; and results of automated CBC analysis[c] within reference intervals.

The study protocol was approved by the Animal Ethics Committee of Murdoch University.

Blood sample collection and processing—The skin over a jugular vein was aseptically prepared for venipuncture in all dogs. A 21-gauge butterfly catheter was placed in the prepared vein through a stab incision and connected to allow blood collection (4.5 mL) into each of 4 evacuated tubes containing 3.2% trisodium citrate. The first tube in each set was discarded, and the remaining 13.5 mL of citrated whole blood was used for the experiment.

Each citrated whole blood sample was diluted with 6% HES 130/0.4,[d] 10% HES 200/0.5,[e] and saline solution at a ratio of 1:9 (0.2 mL of solution to 1.8 mL of whole blood) and 1:3 (0.5 mL of solution to 1.5 mL of whole blood) and was maintained at 38.0°C in a water bath. Within 2 hours after sample collection, Ct for each diluted blood sample was measured with the platelet function analyzer and collagen and ADP–coated cartridges in duplicate. When duplicate measurements had a covariance > 15%, or when the analyzer indicated an error such as flow obstruction, a new sample at the same dilution was created, and a repeated measurement was made. Results that had a covariance > 15% were then excluded from analysis.

Statistical analysis—Closure time was used as the response variable of interest, and values were assessed for a normal distribution with the Shapiro-Wilk test. Because the test revealed that the values were not normally distributed, Cts were reported as median (IQR). Reciprocal transformation resulted in a normal distribution, so 1/Ct was used for the response variable in statistical tests. A generalized linear mixed model was developed by use of statistical software,[f] with fixed effects of solution (3 levels) and dilution (3 levels) and a random effect for dog. When a significant ($P < 0.05$) interaction of solution and dilution was identified, post hoc comparisons were performed across solutions and dilutions with the least squares means method and Bonferroni correction, with values of $P < 0.005$ considered significant.

Results

Blood samples were successfully collected from all 10 healthy dogs and were processed in accordance with the experimental protocol. The median predilution PCV was 48% (IQR, 42% to 50%), and platelet count was 276 $\times 10^9$ cells/L (IQR, 192 $\times 10^9$ cells/L to 440 $\times 10^9$ cells/L). The PCV and the platelet count of all blood samples diluted with saline solution remained > 32% and > 86 $\times 10^9$ cells/L, respectively (**Table 1**). One blood sample diluted 1:9 with saline solution was excluded from statistical analysis because of a dilution error.

A dilutional effect on Ct was identified for the 1:3 dilution, compared with the 1:9 dilution, for each type of solution tested, but only samples diluted with HES 200/0.5 had an increase in Ct beyond a dilutional effect at the 1:3 dilution ($P = 0.002$). No effect of HES or dilution on Ct was evident for the 1:9 dilutions (**Table 2**). Fourteen of the 140 (10%) measurements needed repeated measurement because of a high covariance or

Table 1—Median (IQR) PCV and platelet count for 1:3 and 1:9 dilutions with saline (0.9% NaCl) solution of whole blood samples from 10 healthy dogs.

Dilution	PCV (%)	Platelet count ($\times$ 10^9 cells/L)
None	48 (42–54)	276 (192–440)
1:9	41 (30–50)	243 (100–288)
1:3	36 (32–42)	213 (86–326)

Table 2—Median (IQR) platelet Ct for 1:3 and 1:9 dilutions with saline solution, HES 130/0.4, and HES 200/0.5 of whole blood samples from 10 healthy dogs.

Dilution	0.9% NaCl	HES 130/0.4	HES 200/0.5
None	70 (66–73)[A]	70 (66–73)[A]	70 (66–73)[A]
1:9	71 (62–83)[A]	83.5 (71–91)[B]	80.5 (73–84)[A]
1:3	92 (85–100)[a,B]	101 (94–112)[a,b,C]	125 (118–140)[b,B]

[a,b]Within a row, values with different superscript letters differ significantly ($P \geq 0.005$; Bonferroni corrected). [A–C]Within a column, Cts with different superscript letters differ significantly ($P \geq 0.005$; Bonferroni corrected).
The reference interval used for Ct was 52 to 86 seconds.[14]

machine error; however, no sole measurement was repeated more than twice.

Discussion

The present study revealed that a 1:3 dilution of canine blood with HES 200/0.5 but not HES 130/0.4 led to an increase in platelet Ct beyond a dilutional effect. This in vitro dilution was chosen to mimic the dilution achieved in vivo through IV administration of a fluid bolus of 30 mL/kg, ignoring redistribution and excretion in dogs. Findings suggested that HES 130/0.4 had less of an adverse effect on platelet function than did HES 200/0.5. We also found that a 1:3 dilution resulted in an increase in Ct beyond the upper reference limit, no matter which solution was used.

The increase in Ct associated with dilution with any solution can be explained by a decrease in PCV and platelet count. A negative relationship between Ct and PCV in dogs has been described.[14,16] Changes in Hct can affect platelet function because of changes in blood flow dynamics altering platelet interaction with the endothelium,[23] which may also hold true for contact with the aperture of the platelet function analyzer used, as well as a decrease in ADP release from erythrocytes.[24] A decrease in platelet count may have also contributed to the increase in Ct in the diluted samples in the present study, which has been identified in previous studies[14,16] involving dogs. This effect may have been enhanced by dilution of von Willebrand factor.[25]

Several theories may explain the mechanism by which HES 200/0.5 dilution caused platelet dysfunction in the present study, including binding of von Willebrand factor,[26,27] HES molecules coating platelet surfaces, and a decrease in expression of platelet surface protein integrin αIIbβ3, which all may reduce platelet adhesion and aggregation.[28] An in vitro study[18] of human platelet function revealed a decrease in expression of integrin αIIbβ3 receptor with dilutions involving HES 200/0.5 but not HES 130/0.4.[18] The differences identified between HES 200/0.5 and HES 130/0.4 might be related to solution-specific pharmacological

properties, in particular molecular weight and molar substitution.[27,29]

The molecular weight of an HES solution is the mean manufactured molecular weight of the polydispersed solution, and the molar substitution is the number of hydroxyl groups substituted with hydroxyethyl groups on the glucose subunit of HES molecules.[29] Hydroxyethyl starch solutions of higher molecular weight and molar substitution have a slower rate of degradation and excretion in vivo than do other HES solutions, and this phenomenon has commonly been cited as the main reason that treatment with these solutions causes greater platelet dysfunction.[29,30] However, these pharmacokinetic properties may not be relevant to in vitro studies. It is possible that cleaving of HES molecules by amylase may still occur in vitro, as it does in vivo, decreasing the HES molecular weight within the sample; therefore the rate of degradation may still be relevant. Some evidence exists that high molar substitution can considerably affect the degree of whole blood coagulation in vitro, independent of molecular weight.[31] But there is little evidence regarding the effect of differences in molecular weight and molar substitution, independent of each other, on platelet function specifically in vitro.

Another property related to a slower rate of HES degradation and greater platelet dysfunction is a high C2:C6 ratio[31]; however, this was unlikely to be applicable in the present study given that HES 200/0.5 has a lower C2:C6 ratio (5:1) than does HES 130/0.4 (9:1). The present study did not elucidate the exact mechanisms of HES-induced platelet dysfunction, given that it was designed only to determine whether platelet dysfunction occurred, as detected by the platelet function analyzer.

One additional difference between the 2 HES solutions evaluated was the concentration of HES. The HES 200/0.5 solution had a greater concentration of HES molecules (10%), compared with HES 130/0.4 (6%). The 10% solution was chosen for comparison because it is the solution available for clinical use in Australia. Although it is unknown which solution had the greater number of HES molecules because of the polydiversity among brands and individual bags of solution, HES 200/0.5 may have provided more individual HES molecules, enhancing platelet dysfunction.

Such differences between solutions also create variations in the degree of redistribution in vivo, such as differences in plasma volume expansion, degrees of dilution of blood constituents, and rates of clearance.[32,33] These variations contribute to the challenge of designing in vivo studies, not only in interpreting differences between solutions but also in the ability to select a valid control solution for comparing the effect of dilution, given that crystalloid solutions have a different pattern of distribution. Therefore, in vitro studies such as the present study, although limited by many factors including the absence of any platelet interaction with the endothelium, also have an advantage in that the effect of various solutions can be compared without the complicating factors of redistribution and metabolism. Other complicating factors common to in vivo studies designed to assess the effect of HES solutions on platelet

function include variability in doses used, differences in the disease status of patient populations, and choice of method of platelet function analysis, making comparisons of findings across studies difficult.

We chose 1:9 and 1:3 dilutions with the aim of indirectly comparing results of the present study with those of another study,[6] which demonstrated that whole blood diluted with HES 670/0.75 led to an increase in Ct beyond a dilutional effect when a 1:3 but not 1:9 dilution was used. The 1:9 dilution mimicked a 10 mL/kg bolus dose and the 1:3 dilution mimicked a 30 mL/kg bolus dose administered IV to a dog with an estimated blood volume of 90 mL/kg (ie, euvolemia). The effect of the same in vitro dilutions on Ct in dogs was investigated in another study[22] involving an HES solution of low molecular weight and molar substitution, HES 130/0.42, which has some differences in properties compared with HES 130/0.4. A significant increase in Ct was identified with HES 130/0.42 diluted 1:3 with canine whole blood beyond a dilutional effect, which differs from our findings, despite the 2 solutions being very similar. Several factors could account for this difference. Hydroxyethyl starch 130/0.4 is a waxy, maize-based colloid, whereas HES 130/0.42 is a potato-based colloid. The 2 solutions also differ in molar substitution, which may account for the difference in Ct. A conservative value of $P < 0.005$ was used for pairwise comparisons made in our study to reduce type I error, which may have precluded detection of more differences than were identified. Differences in study subjects and subject numbers as well as a lower predilution Ct in our study may have also contributed to different results. For comparison, an in vitro study[3] in humans compared the effect on coagulation between these 2 solutions through use of thromboelastography and platelet aggregometry and found no difference between the solutions, but HES 130/0.4 and HES 130/0.42 have not been compared directly in dogs.

The clinical relevance of the effect of HES solutions with low molecular weight and molar substitution on platelet function in humans remains controversial despite the abundance of literature on the topic. Early in vitro studies indicated that blood samples diluted with HES 130/0.4 caused less platelet dysfunction as measured through various techniques such as platelet aggregometry,[4,34] Ct analysis,[4,5,18] and flow cytometry,[18] compared with that caused by HES solutions of higher molecular weight and molar substitution. However, several clinical trials[1,20,21] comparing the effects of HES 130/0.4 with HES 200/0.5 on blood loss and transfusion requirements in orthopedic surgery, cardiopulmonary bypass patients, and intensive care patients have yielded conflicting evidence, compared with the in vitro studies. Meta-analyses and literature reviews[1,20,21] have shown that no conclusion can be made regarding the benefit of administration of HES 130/0.4 versus HES 200/0.5 or crystalloid solutions in humans because of the small sample sizes and the quality of these human trials and because several reports were retracted for scientific misconduct. In addition, although the manufacturers of HES 130/0.4 have recommended a dose limit of 50 mL/kg/d, the European Society of Intensive Care Medicine released a consensus statement, in which no dose recommendations for HES 130/0.4 could be made given the minimal evidence for its safety regarding blood loss, acute kidney injury, and risk of death.[35] Most recently, the Surviving Sepsis Campaign consensus statement has recommended against the use of HES solutions in septic human patients because of the lack of clear benefit and evidence of harm caused to patients,[36] but large clinical trials have yet to be performed in veterinary medicine.

The present study demonstrated that dilution of canine blood samples 1:3 with saline solution, HES 130/0.4, and HES 200/0.5 led to an increase in Ct attributable to a dilutional effect alone. Hydroxyethyl starch 200/0.5, however, led to an increase in Ct beyond a dilutional effect when diluted 1:3 with blood, consistent with platelet dysfunction. Interestingly, we found that HES 130/0.4 did not cause platelet dysfunction at this same dilution, which suggests that this solution may be a superior choice when platelet dysfunction is of concern. However, in vivo studies are warranted to investigate the risk of clinical use of this solution in dogs.

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