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Effect of Hemodilution *In Vitro* with Hydroxyethyl Starch on Hemostasis

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Background: Hydroxyethyl starch (HES) solutions are used for volume expansion during surgery. We aimed to investigate how 6%HES 130/0.4 affects hemostasis.





Material/Methods: Blood samples were collected from 12 healthy adult volunteers, diluting with 6%HES 130/0.4 (HES group) or Ringer lactate solution (RL control group). The hemodilution ratio (HR) of citrated blood volume to plasma substitute volume was 10: 0 (undiluted), 10: 2, 10: 4, and 10: 6. Clotting factors activity was measured. Thrombin generation was monitored. Platelet function was analyzed.

Results: 1) Activity of coagulation factor was decreased with increasing HR compared to undiluted baseline, and the activity of FVIII was significantly decreased in HES vs. RL. 2) Calibrated automated thrombography (CAT) results showed HES extended lag time, time to peak (t_{peak}), start tail, and decreased peak of thrombin generation. Although lag time and t_{peak} were significantly prolonged in HES vs. RL, endogenous thrombin potential (ETP) did not change. 3) Flow cytometric (FCM) analysis showed that HES reduced platelet phospholipids serine (PS) vs. baseline and RL. 4) HES significantly decreased antithrombin activity (AT: A) of the anticoagulant system with increasing HR vs. baseline and RL. 5) For fibrinolytic system, HES did not affect fibrinogen degradation products (FDP) and D-dimers (D-D) vs. baseline, or α₂-antiplasmin (α₂-AP) vs. RL.

Conclusions: By reducing FVIII activity and platelet PS expression, HES interfered with PS combining to FXIa, FVIIIa, and FVa, which affected the acceleration and explosion stage of thrombin. The decreased velocity and peak of thrombin generation delays and reduces clot formation. Combined 6%HES 130/0.4 decreased anticoagulant activity and may have clinical utility.

MeSH Keywords: **Blood Platelets • Hemodilution • Hemostasis • Hydroxyethyl Starch Derivatives**

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Background

Maintaining adequate circulating blood volume is important during major surgery, such as orthopedic surgery, cardiac surgery, and urologic surgery [1]. Colloidal plasma substitutes are widely used for intravascular volume expansion, especially for the prevention and treatment of perioperative hypovolemia, such as HES solutions among colloids [2,3]. The administered colloids interfere with hemostasis, resulting in high risk of bleeding or an adverse effect through the degree of hemodilution and through the influence on platelet function, coagulation system, and the fibrinolytic system [4,5]. Previous report suggested that extensive hemodilution can lead to a complex coagulation disorder [6].

A systematic review showed that HES, which is a type of plasma volume expander, has an undesirable effect on hemostasis as measured by thrombelastography (TEG) [7]. For HES, hydroxyethyl groups can be attached to the C2, C3, and/or C6 of the glucose moieties of amylopectin [2,5,8]. It is well known that HES interferes with blood coagulation according to its molecular weight (kDa) and the degree of substitution (hydroxyethyl groups/10 u of glucose) [2,8]. And the volume expansion varied with the diluting level of HES solutions [2]. Several studies suggested that HES negatively affects coagulation factor VIII (FVIII) and von Willebrand factor (vWF) and impairs platelet function [2,9,10]. Previous studies supported that hemodilution with the latest generation of HES solutions (6%HES 130/0.4) inhibited platelet function and affected coagulation to a lesser extent in comparison with a first- and second-generation HES (HES 450/0.7 and HES 200/0.5, respectively) [8]. In addition, researchers reported that colloid liquid of 6%HES 130/0.4 can activate the fibrinolytic system and alleviate hypercoagulability state in patients with septic shock, just as the Ringer lactate (RL) does [11]. Studies have demonstrated that RL has no influence on coagulation after fluid administration during major surgery [12,13].

Coagulation is an integrated process in which the signals of procoagulant factors and anticoagulant factors are balanced [14]. The coagulation cascade is a series of proteolytic reactions of factors resulting in thrombin generation (TG), leading to clot formation (converting fibrinogen to fibrin) [15]. Fibrinogen (Fg) has been described as the earliest coagulation factor [16]. TG starts by forming the tissue factor (TF)-activated factor VII (FVIIa) complex at the vascular wall of injury site and activates FX, then FVa and FXa, to form the prothrombinase complex, which is a process of positive feedback leading to thrombin (FIIa) generation, followed by the activation of factor V (FV), factor VIII (FVIII), and factor XI (FXI) to accelerate its formation [17,18].

It is well known that blood flow maintenance depends on the proper balance of hemostasis and fibrinolysis. Accompanied by the coagulation pathway, the protein C pathway and the

fibrinolytic pathway, which are both anticoagulant pathways, are activated to avoid excessive clot formation or thrombosis [18]. Activated protein C (PC) inactivates FVa and FVIIIa, thereby limiting further thrombin formation in the presence of protein S [18]. The reduction of or deficiency in antithrombin activity (AT: A) caused by hemodilution results in sustained thrombin activity and thrombin generation [6]. Because fibrin serves as the substrate for plasmin, α_2 -antiplasmin (α_2 -AP) participates predominantly in inhibiting fibrinolysis [19]. Fibrinolysis can be verified by elevated D-dimers (D-D) levels and elevated fibrinogen degradation products (FDP) [20].

Platelets play a vital role in thrombosis by forming a hemostatic plug. Researchers reported that circulating platelets were located to the subendothelium of a damaged vessel wall, then glycoprotein IIb/IIIa (GpIIb/IIIa) is induced to activate by the clustering of GpIIb/IIIa receptor on the surface of the platelets, with subsequent binding of fibrinogen (Fg) or vWF to form an unstable platelet plug and gradually becomes a stable platelet-fibrin plug resulting from the increasing procoagulant activity and thrombin generation [21]. Platelets are involved in the total hemostasis process by adhesion to vessel wall, activation, granule secretion, aggregation (interaction of platelets), and stabilization [22]. The soluble adhesion molecule, P-selectin (CD62-P), located on the α -granule membrane of platelets, could be a clinically useful marker for platelet activation status during thrombosis formation [21]. Phosphatidylserine (PS) forms part of the plasma membrane of platelets [21].

As basic plasmatic coagulation tests, prothrombin time (PT) and activated partial thromboplastin time (APTT) are used to investigate clot formation time when only around 5% of all physiologically relevant thrombin is generated; therefore, PT and APTT are not necessarily good indicators of thrombin activity [17,23]. Thrombin generation assay (TGA) has been developed to assess the coagulation of whole blood [24], or platelet-rich plasma (PRP) and platelet-poor plasma (PPP) [14], in bleeding and thrombotic disorders [25]. The thrombogram or thrombin generation curve can be readily obtained through calibrated automated thrombography (CAT), which requires dedicated reagents to trigger coagulation, as well as commercially available fluorometers equipped with dedicated software to record and calculate data [25,26]. Four major parameters can be assessed in this automated version of TGA: lag time, time to peak, thrombin peak height (peak), and endogenous thrombin potential (ETP) [23].

Although 6%HES 130/0.4 has a lower molecular weight and a degree of substitution that interferes little with hemostasis and has fewer adverse effects, the detailed effect on coagulation, platelet function, and fibrinolysis remain to be delineated for clinical application. In the present study, we aimed to explore the relevant mechanism by which 6%HES 130/0.4

effects hemostasis involving blood coagulation, platelet function, anticoagulation, and fibrinolytic activity.

Material and Methods

Subject

With the approval of the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, we enrolled 12 healthy volunteers aged 28 to 53 years who did not take any anticoagulant or anti-platelet medication during the last 2 weeks. All volunteers gave written informed consent. The subjects were fasted for 8 h prior to the collection of blood samples. Exclusion criteria were pre-existing bleeding disorders, renal dysfunction, and liver disease.

Blood collection

Blood was withdrawn into Vacuette™ tubes (Becton, Dickinson, and Company, USA) containing 0.129 mol/L trisodium citrate (9: 1 vol/vol), which was drawn from an antecubital vein by venipuncture without stasis using a 19-gauge butterfly needle. The first 3 mL were always discarded. Each blood sample was diluted with 6%HES 130/0.4 (Fresenius Kabi Global, Germany) or Ringer's lactate solution (RL) (Shanghai Baite Medical Product Co., Ltd, China). The hemodilution ratios (HR) of citrated blood volume to plasma substitute volume were 10: 2, 10: 4, and 10: 6. We defined 10: 0 (undiluted) as the baseline value. The study was designed to analyze the difference in parameters of the HES group compared with those in the RL group, and to compare these with undiluted baseline values (HR=10: 0). The appropriate amount of blood was repeatedly reverse pipetted to fill into pre-warmed (37°C) polypropylene tubes containing HES or RL to prevent cooling of the blood and expander mixture, which might affect blood coagulation. Mixing was performed carefully by filling and half-emptying the pipette 3 times, and the same procedure was performed with native blood. All incubations were performed for 5 min.

Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) preparation

Blood samples treated with trisodium citrate were centrifuged at 500 rpm for 2 min at room temperature, and the plasma supernatant was collected as PRP. Platelet count (PLC) and platelet volume (PLV) were analyzed using a Beckman Coulter LH-750 device (Beckman Coulter, USA).

PRP continued to be centrifuged at 3000 rpm for 15 min, then the upper 2/3 of plasma supernatant was obtained and centrifuged again at 3000 rpm for 15 min, and the upper 2/3 volume of plasma supernatant was eventually collected as PPP.

The PRP samples were diluted with autologous PPP to produce a platelet density of $150 \times 10^9/L$. PRP samples were frozen at $-80^\circ C$ to be further analyzed.

The PT, APTT, thrombin time (TT), fibrinogen, factors II, V, VII, VIII, IX, X, XI and XII, protein C, vWF, FDP, D-D, α_2 -AP, and antithrombin were analyzed using an MDA II analyzer (BioMérieux UK, Ltd., Basingstoke, UK) according to the manufacturer's instructions.

Flow cytometric analysis

Flow cytometric analysis was performed to assess the expression of phosphatidylserine (PS), CD62P, and GPIIb/IIIa on the active platelets, which is necessary and sufficient to account for platelet surface thrombin generation. The whole-blood sample was diluted (1: 5) in phosphate-buffered saline (100 mM of sodium phosphate, pH value of 7.3, and 0.145 M of NaCl) to inhibit contact between individual platelets. Each sample was further divided into 2 aliquots for fluorescent staining. To evaluate the extent of platelet reactivity, one aliquot was incubated with the strong platelet agonist thrombin receptor activator peptide 6 (TRAP, 25 μM , SFLLRN, Bachem AG, Bubendorf, Switzerland) and the other aliquot was stained without platelet stimulation. After 30 min of incubation with phycoerythrin-conjugated monoclonal antibody against human platelet PS, annexin V, CD62P, and GPIIb/IIIa at room temperature in the dark, samples were fixed in 10% paraformaldehyde (pH 7.3) at 4°C. In each experiment, cellular autofluorescence was determined, and isotype and compensation control were performed. Fluorescence was measured with a FACSCalibur™ flow cytometer and analyzed with CellQuest-Pro™ software (Becton Dickinson Immunocytometry Systems).

Thrombin generation

Thrombin generation in PRP was measured by CAT method, as previously reported [27,28]. The 2.5 mM fluorogenic substrate and 100 mM calcium solution (FluCa) (Thromboscope BV, The Netherlands) containing ZGGR-AMC and Fluo-buffer were prepared before the experiment; they released the fluorescent AMC after splitting by thrombin, as detected by use of a Fluoroskan Ascent FL (Thermo Eletron and Fisher Scientific, Helsinki, Finland) device equipped with a dispenser. Fluorescence intensity was detected with an excitation filter at 390 nm and an emission filter at 460 nm.

After immersion in a water bath at 37°C for 10 min, 80 μL of PRP or PPP samples was added per well to an Immulon 2HB 96-well plate (Thromboscope BV, Netherlands). We added 20 μL PPP reagent or PRP reagent or thrombin calibrator (Thromboscope BV, The Netherlands) to carry out the fluorogenic reaction. Each sample and calibrator was set as 3 repeated wells, followed by the addition of 20 μL of FluCa per well to initiate the coagulation process and measure thrombin activity. The data were

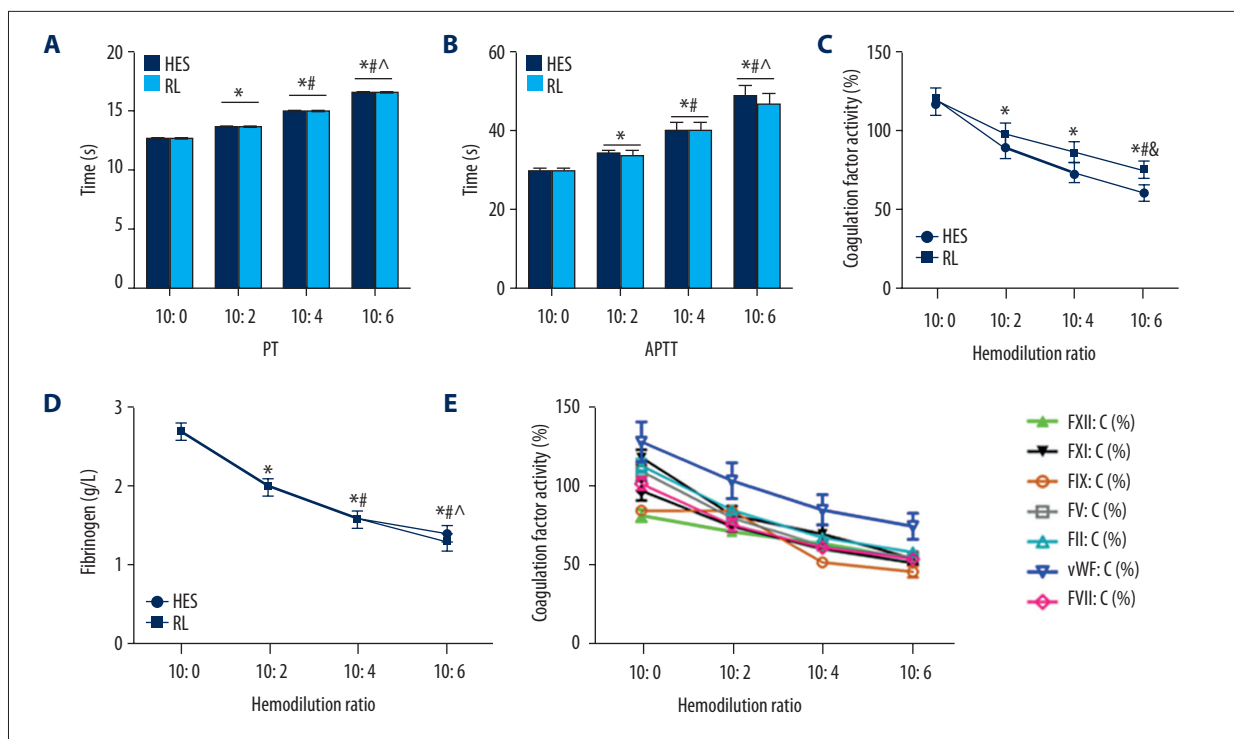


Figure 1. PT and APTT varied with the hemodilution ratio of 6%HES 130/0.4 and RL 10: 0, 10: 2, 10: 4, and 10: 6 was defined as the hemodilution ratios (HR) of citrated blood volume to plasma substitute volume. (A) PT, prothrombin time (s); (B) APTT, activated partial thromboplastin time (s); (C–E) FVIII: C, FVII: C, FXII: C, FXI: C, FIX: C, FV: C, FX: C, FII: C (%) means clotting activity of each blood coagulant factor; vWF – von Willebrand factor; Fg (g/L) – fibrinogen. * Indicates $p < 0.05$ vs. undiluted baseline values at HR=10: 0; # indicates $p < 0.05$ versus HR=10: 2; ^ indicates $p < 0.05$ vs. HR=10: 4, in either HES group or RL group; & indicates $p < 0.05$ compared with RL at the same dilution ratio.

analyzed using Thrombinoscope™ software (Thrombinoscope BV, The Netherlands) to determine ETP, peak, time to peak, lag time (LT, defined as the moment that the signal deviates by more than 2 standard deviations from the horizontal baseline), and start tail for each sample.

Statistical analysis

Statistical analysis was performed with SPSS 18.0 software (SPSS, Chicago, IL, USA) and distribution of data was confirmed. All data are presented as mean ±SD in absolute values. Differences from the baseline were evaluated by paired t-tests. Values were considered significant at $p < 0.05$.

Results

Hemodilution with 6%HES 130/0.4 inhibited blood coagulation system compared with RL

PT and APTT were significantly ($P < 0.05$) prolonged at different dilution ratios of citrated blood to HES (HR=10: 6, 10: 4 and 10: 2) when they were compared with the baseline value

(HR=10: 0/undiluted). However, we found no significant difference between the HES and RL groups ($P > 0.05$). Higher HR was associated with longer PT and APTT (Figure 1A, 1B). PT and APTT were in the normal range when HR=10: 2 and 10: 4, but it was close to the upper limit of the baseline value when HR=10: 6.

The coagulation factor activity of FVIII, FVII, FXII, FXI, FIX, FV, FX, FII, vWF, and fibrinogen (Fg) were significantly decreased with increasing dilution ratio (HR=10: 2, 10: 4, and 10: 6) in comparison with HR=10: 0 in the HES group. We found no significant difference when comparing the HES group with RL group in each HR, except that FVIII: C was significantly decreased at HR=10: 6 in the HES group compared with the RL group ($P < 0.05$) (Figure 1C–1E).

The parameters determined by CAT showed that the peak thrombin generation gradually decreased when the dilution ratios of citrated blood to HES was 10: 2, 10: 4, and 10: 6 vs. 10: 0 (Figure 2A); however, lag time and tpeak of thrombin generation were prolonged at HR=10: 4 and 10: 6 compared with baseline values in the HES group but not in the RL group. Start tail was increased when compared with the baseline

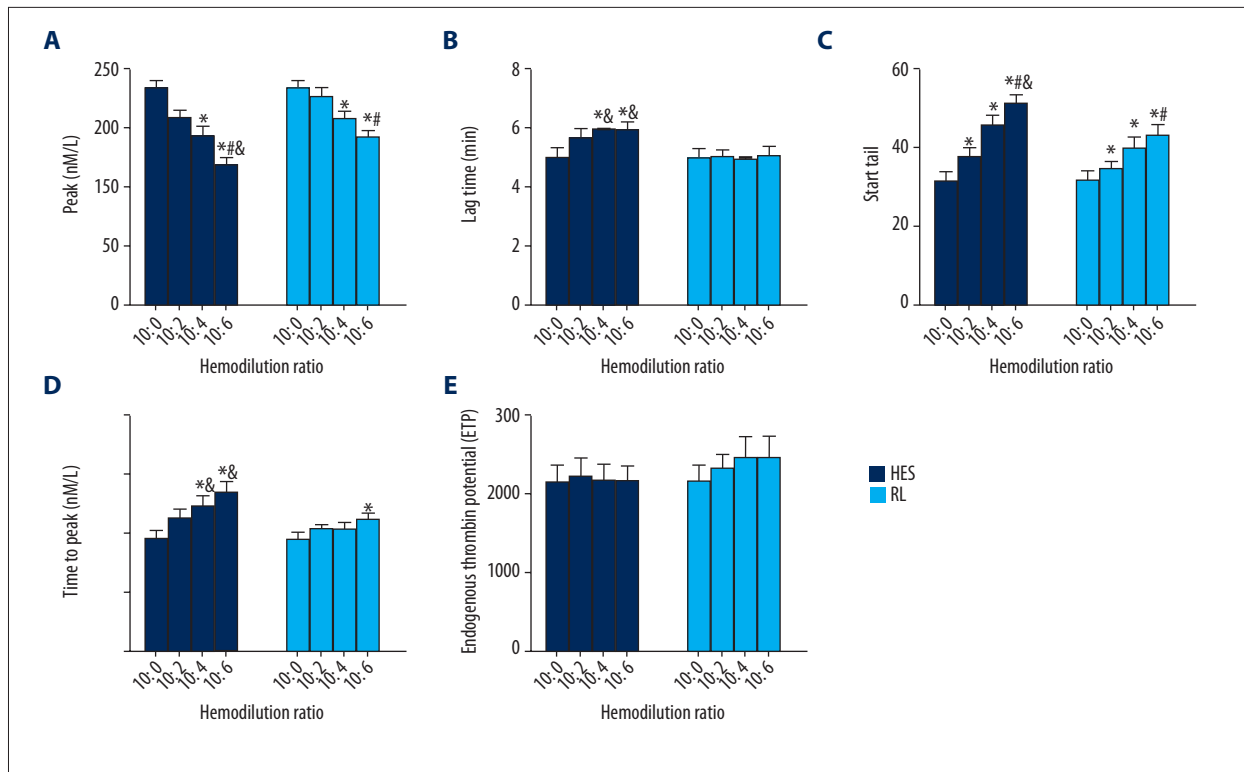


Figure 2. Parameters of thrombin generation measured by calibrated automated thrombogram (CAT) varied with the hemodilution ratio of 6% HES 130/0.4 and RL. (A) Peak. (B) Lag time. (C) Start tail. (D) Time to peak. (E) Endogenous thrombin potential (ETP). * Indicates $p < 0.05$ vs. undiluted baseline values at HR=10: 0; # indicates $p < 0.05$ vs. HR=10: 2; ^ indicates $p < 0.05$ vs. HR=10: 4; & indicates $p < 0.05$ compared with RL at the same dilution ratio.

values in both groups (Figure 2B–2D). Furthermore, peak at HR=10: 6 was lower than that at HR=10: 2 and HR=10: 4 in the HES group vs. that in the RL group. Start tail at HR=10: 6 and HR=10: 4 was significantly longer than that at HR=10: 2 in the HES group (Figure 2B). However, endogenous thrombin potential (ETP) was not affected by HES or RL at the 3 dilution ratios compared with baseline, or compared between the HES and RL groups (Figure 2E).

Hemodilution with 6% HES 130/0.4 negatively affected platelet function compared with RL

The platelet count (PLC) decreased with increasing dilution ratio in the HES and RL groups, which showed statistical significance at HR=10: 2, 10: 4, and 10: 6 vs. HR=10: 0 ($P < 0.05$). PLC was significantly lower at HR=10: 4, 10: 6 vs. HR=10: 2 ($P < 0.05$) (Figure 3A). However, platelet volume (PLV) had no obvious variation among all the dilution ratios in both groups (Figure 3B). The expression of platelet phosphatidylserine (PS) decreased at HR=10: 2, 10: 4, and 10: 6 vs. HR=10: 0 in the HES group ($P < 0.05$), but PS did not significantly change in the RL group, and PS was significantly lower at HR=10: 4 and 10: 6 in the HES group vs. the RL group ($P < 0.05$) (Figure 3C). In addition, the expression of platelet CD62-P (P-selectin) and

GPIIb/IIIa (CD41a) was little changed, with no statistical difference in either group (Figure 3D, 3E).

Hemodilution with 6% HES 130/0.4 slightly inhibited the anticoagulation system compared with RL

After hemodilution with HES and RL *in vitro*, antithrombin activity (AT: A) and protein C activity (PC: A) significantly decreased with increasing dilution ratio (HR=10: 2, 10: 4, 10: 6) in the HES and RL groups, compared with the baseline values. Importantly, HES can significantly reduce the level of AT: A without affecting PC: A when compared with RL at HR=10: 2 and 10: 4 ($P < 0.05$) (Figure 4).

Hemodilution with 6% HES 130/0.4 inhibited the fibrinolytic system compared with RL

Fibrin degradation product (FDP) and D-dimers (D-D) did not change significantly at each dilution ratio in the HES group compared with the baseline values (Figure 5A, 5B), and D-D showed significant differences at HR=10: 4 and 10: 6 in the HES group compared with the RL group ($P < 0.05$). However, α_2 -antiplasmin (α_2 -AP) was down-regulated with increasing dilution ratios, and was significantly lower at HR=10: 2, 10: 4, and 10: 6 than at HR=10: 0 in the HES and RL groups ($P < 0.05$),

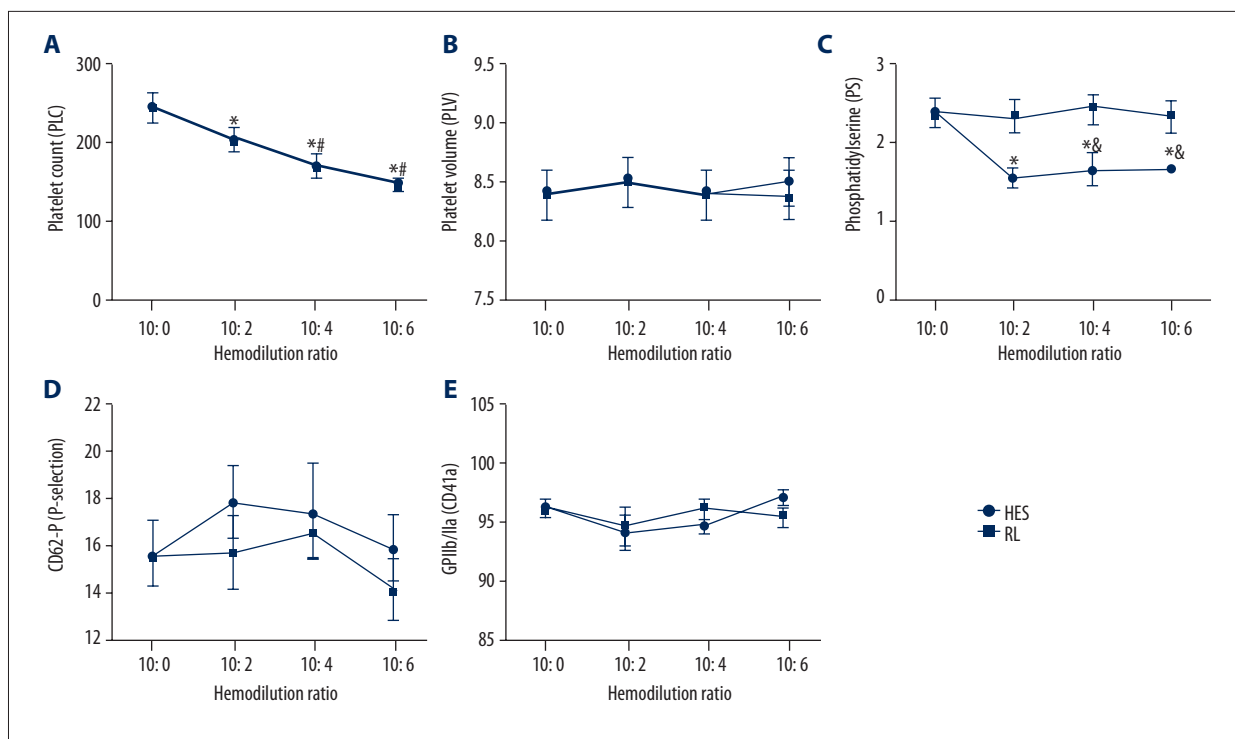


Figure 3. Platelet related protein expression measured using flow cytometry varied with the hemodilution ratio of 6%HES 130/0.4 and RL. (A) Platelet count (PLC). (B) Platelet volume (PLV). (C) Phosphatidylserine (PS). (D) CD62-P (P-selection). (E) GpIb/IIa (CD41a). * Indicates $p < 0.05$ vs. baseline values, # indicates $p < 0.05$ vs. HR=10: 2; & indicates $p < 0.05$ compared with RL at the same dilution ratio.

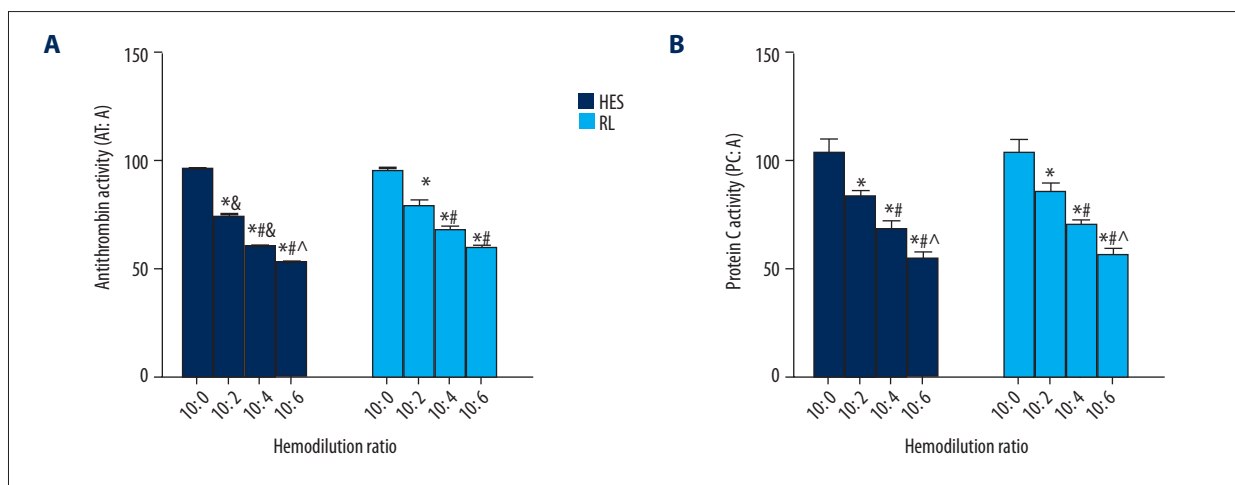


Figure 4. Anticoagulation system varied with the hemodilution ratio of 6%HES 130/0.4 and RL. (A) Antithrombin activity (AT: A); (B) Protein C activity (PC: A). * indicates $p < 0.05$ vs. baseline values at HR=0; # indicates $p < 0.05$ vs. HR=10: 2; ^ indicates $p < 0.05$ vs. HR=10: 4 in both HES group and RL group; & indicates $p < 0.05$ compared with RL at the same dilution ratio.

but showed no significant difference when compared between the 2 groups (Figure 5C). Thrombin time (TT) was significantly shortened at different dilution ratios compared with the baseline values in the HES group, and TT showed significant differences at all 3 dilution ratios (HR=10: 2, 10: 4, 10: 6) in the HES group compared with the RL group ($P < 0.05$) (Figure 5D).

Discussion

Major surgery often results in serious blood loss. Volume replacement with colloids can compensate for hypovolemia, but hemostatic defects or coagulopathy can occur after massive transfusion and hemodilution. Many factors contribute to

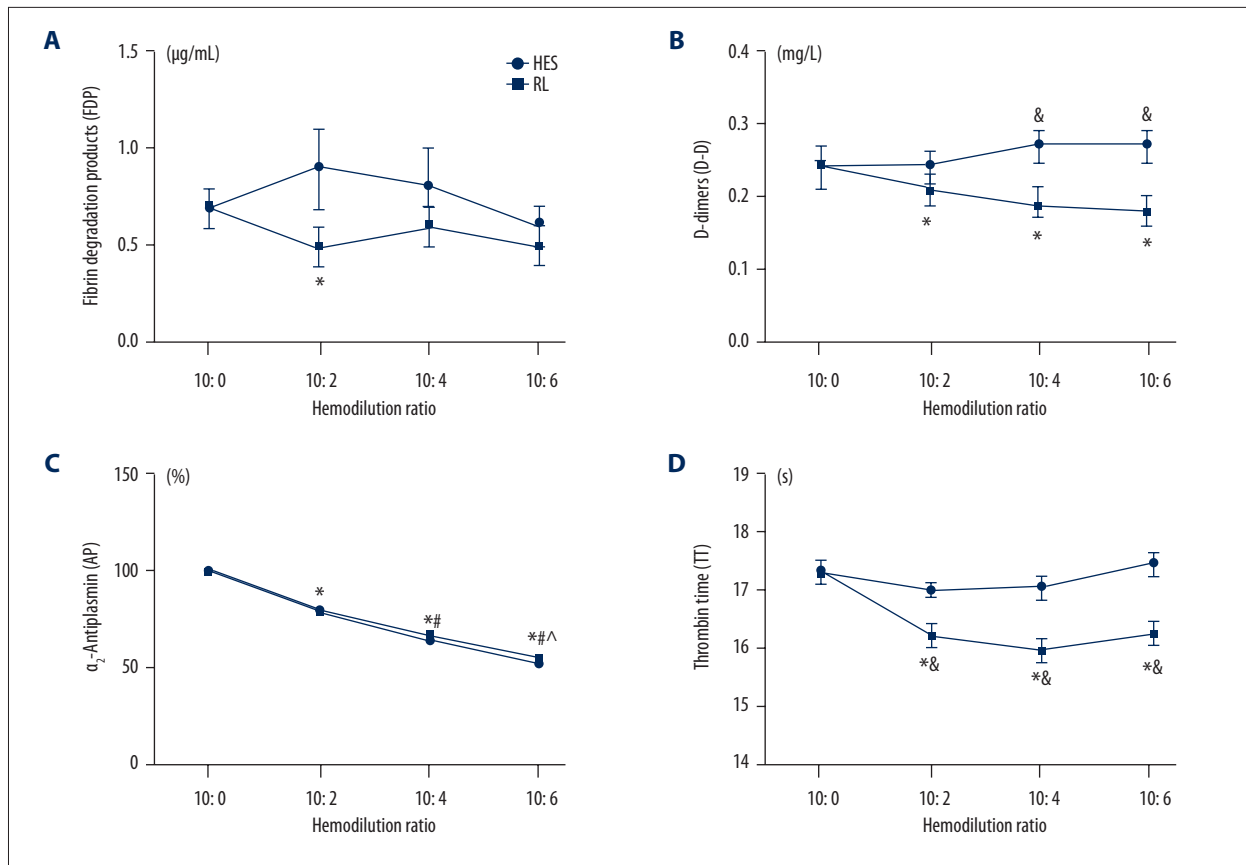


Figure 5. Fibrinolytic activity varied with the hemodilution ratio of 6%HES 130/0.4 and RL. (A) Fibrin degradation products (FDP). (B) D-dimers (D-D). (C) α_2 -Antiplasmin (α_2 -AP). (D) Thrombin time (TT). * Indicates $p < 0.05$ vs. baseline values at HR=10: 0; # indicates $p < 0.05$ vs. HR=10: 2; ^ indicates $p < 0.05$ vs. HR=10: 4 in both HES group and RL group; & indicates $p < 0.05$ compared with RL at the same dilution ratio.

abnormal hemostasis, but the mechanism, including procoagulant, anticoagulant, fibrinolytic, antifibrinolytic and platelet elements, is not fully understood. A previous study reported that disrupting the balance between proteolytic and inhibitory reactions in hemostasis and fibrinolysis can result in thrombosis or bleeding conditions, especially through the coagulation, protein C, and fibrinolytic pathways [18].

In the present study, the results acquired by conventional coagulation tests indicated that the coagulation system was significantly impaired with the increase of diluted ratio of 6%HES 130/0.4 solution to citrated blood. On the one hand, the clotting time (PT and APTT) was prolonged with the hemodilution of HES and RL. On the other hand, FVIII: C, FVII: C, FXII: C, FXI: C, FIX: C, FV: C, FX: C, FII: C, vWF: C, and Fg were significantly decreased in both HES and RL groups. Thus, the hemodilution with crystalloid (RL) or colloid (HES) weakened the function of blood coagulation. HES showed a stronger negative influence on coagulation due to the relatively lower activity of FVIII when compared with RL, which reflected the intrinsic effect of HES on coagulation²⁹. The decreased FVIII: C

explains the HES-induced hypocoagulation, as previous studies reported [1,30]. Thrombin generation is the pivotal step of hemostasis [31], and clotting time is not necessarily a good indicator of thrombin activity since <5% thrombin was generated at the moment of clotting, so a thrombin generation curve was produced to monitor thrombin activity [21]. The CAT parameters include peak (representing the highest thrombin concentration that can be generated), time to peak (reflecting the velocity of TG), and endogenous thrombin potential (ETP) (defined as the relative amount of thrombin after weighing the pro- and anticoagulant drivers) [25]. Thrombin generation determined by CAT indicated that 6%HES 130.4 affected the peak value and velocity of thrombin burst. Subsequently, lag time and tpeak was prolonged, especially at the dilution ratio of 10: 4 and 10: 6, with the hemodilution of HES compared with RL, and HES reduced peak and extended start tail at the dilution ratio of 10: 6 compared with RL. However, ETP was not changed by the hemodilution of either HES or RL, indicating the total amount of thrombin generated was almost unchanged. As reported in a previous study, the unchanged ETP could not explain the mechanism of HES-induced dilutional

hypocoagulation; in addition, peak value was related to the occurrence of thrombosis and bleeding [32]. Our results suggest that the hemodilution with 6%HES 130/0.4 *in vitro* slightly impairs coagulation function compared with RL.

It is known that antithrombin (AT) is the principal inhibitor of the coagulation proteases to allow appropriate clot formation while preventing thrombosis [33], and protein C (PC) acts as one of the natural anticoagulants. In our work, the decrease of antithrombin activity (AT: A) may reflect that the hemodilution with 6%HES 130/0.4 had an inhibitory influence on anticoagulation system, and thus had a beneficial influence on coagulation function compared with RL.

Researchers have reported that the activation of platelets at sites of vascular injury resulting in excessive hemostasis play a vital role in the pathogenesis of occlusive arterial disease [33]. Activated platelets can lead to generation of large amounts of thrombin, fibrin formation, and, ultimately, clot formation [31]. In our study, as a simple, inexpensive, and widely available marker of platelet activity, platelet volume (PLV) was not found to be affected by hemodilution with 6%HES 130/0.4 compared with RL. Decreased platelet count (PLC) was affected by the hemodilution of both HES and RL, and showed no difference. The exposure of phosphatidylserine (PS) on the platelet membrane is assumed to be a consequence of platelet activation [34]. In our study, the decreased level of PS may represent the decreased platelet function in hemostasis with the hemodilution of 6%HES 130/0.4 at HR=10: 4 and 10: 6 compared with RL.

Studies have demonstrated that platelets supply factors that support the activation of prothrombin for coagulation [35]. The accelerated stage of thrombin was the process that FVa, FVIIIa and FXIa combined to platelet PS through its respective connexin protein [36]. HES reduced the activity of FVIII: C and the expression of platelet PS, which not only affected the quantity and speed of PS binding to FVIIIa, but also interfered the combination of PS and FVa/FXIa, resulting in prolonged lag time, decreased peak, and delayed generation of thrombin. The procoagulant effectiveness of platelet activation was reduced, thus HES delayed the start of the body's coagulation function and reduced the strength of blood clots.

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Previous research also reported that colloids like HES decrease clot propagation and strength through poor FXIIIa-fibrin polymer crosslinking [30]. Data from the present study strengthen our understanding of the mechanism involved. Although HES affected the peak value and velocity of thrombin burst, only 25–50% of procoagulant substrate and about 60% of platelets are activated at the initial explosion stage of thrombin. With the extension of start tail, there was still a certain amount of thrombin generated in the later explosion stage. Furthermore, HES can reduce the anticoagulant activity, including AT: A, which decreased the inactivation of thrombin. This may be why hemodilution with HES did not affect the ETP of thrombin generation. Researchers reported that GPIIb/IIIa receptor activity was related to fibrin clot strength and thrombin generation [38]. However, the present study found that hemodilution with HES *in vitro* did not affect the expression of GPIIb/IIIa, showing that the effect on blood clot strength and thrombin was irrelevant to GPIIb/IIIa.

In our study, FDP and D-D were not affected by the hemodilution of either HES or RL by comparing them with the undiluted baseline values. α_2 -AP was down-regulated with the hemodilution of both HES and RL, showing no difference. Thus, fibrinolytic function was not affected by HES compared with RL.

Our study utilized the method of *in vitro* hemodilution to explore the effect of 6%HES 130/0.4 on blood coagulation function and its related mechanisms, mainly focused on the coagulation system, platelet function, anticoagulation system, and fibrinolytic system function. However, it is still unclear how HES reduced the expression of platelet PS, and whether the capacity of PS binding to activated coagulation factor changed. These and other questions need to be answered by further studies.

Conclusions

The intrinsic effect of hemodilution with 6%HES 130/0.4 *in vitro* on coagulation function is mild compare with RL. In the clinical application dosage, HES will not increase the risk of hemorrhage tendency if the patients' coagulation function, platelet function, anticoagulation function, and fibrinolytic function are normal.

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