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BRIEF REPORT

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Decreased circulating levels of von Willebrand factor after intravenous administration of a rapidly degradable hydroxyethyl starch (HES 200/0.5/6) in healthy human subjects

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Abstract *Objective*: Impairment of haemostasis has been described with slowly degradable medium molecular weight hydroxyethyl starch (MMW-HES), whereas rapidly degradable MMW-HES is generally considered to have no important effects on blood coagulation. This study was undertaken to investigate the effects of a rapidly degradable MMW-HES plasma substitute on primary haemostasis and blood coagulation in human subjects.
Design: Randomised, cross-over study.
Setting: Research unit of a university hospital.
Participants: Nine healthy, adult male volunteers.
Interventions: A 60-min intravenous infusion of 1 l HES 200/0.5/6 (HAES-steril 6 %) or 4 % albumin (control).
Measurement and results: The infusion of HES resulted in decreased circulating levels of von Willebrand factor antigen (from 85 ± 8 % to 59 ± 6 % after HES vs from 80 ± 7 % to 69 ± 8 % after albumin, $p < 0.05$) and ristocetin cofactor activity (from 93 ± 4 to 67 ± 4 % after HES vs from 79 ± 5 to 75 ± 5 % after albumin, $p < 0.01$). This was associated with an impairment of in vitro platelet function as determined with the PFA-100 platelet function analyser (closure time with collagen/epinephrine from 120 ± 7 to 159 ± 14 s after HES vs from 121 ± 7 to 137 ± 10 s after albumin, $p < 0.05$; with collagen/ADP from 88 ± 3 to 116 ± 9 s and from 103 ± 4 to 114 ± 7 s after HES and albumin, respectively, $p = 0.01$).
Conclusions: The infusion of 1 l of HES 200/0.5/6 in healthy human subjects results in moderately decreased plasma levels of von Willebrand factor associated with impairment of platelet function.

Keywords Hydroxyethyl starch · Plasma substitute · Haemostasis · Von Willebrand factor

Introduction

Hydroxyethyl starches (HES) are widely used as plasma substitutes because they carry no risk of transmitting viral- or other transfusion-related disease and are less expensive than plasma products. Different types of HES are characterised by the mean molecular weight (MW), the degree of substitution of hydroxyethyl for hydroxyl groups and by the C2/C6 hydroxyethylation ratio. In the United States, the predominantly used HES has an average MW of 480 and a substitution ratio of 0.7 (HES 480/0.7, Hetastarch). Significant bleeding complications have been described after treatment with HES 480/0.7 and are associated with decreased levels of circulating factor VIII and von Willebrand factor (vWF) [1, 2]. HES with medium MW (MMW-HES) is commonly used in Europe. After repeated use of MMW-HES that is slowly degradable (high substitution ratio, high C2/

C6 ratio) a marked decrease of plasma levels of vWF was observed, probably due to accumulation of HES molecules with a high MW [3]. In contrast, the effects of highly degradable MMW-HES with low substitution and low C2/C6 ratio (HES 200/0.5/6) on haemostasis, although generally considered to have no important influence [3], have not been characterised precisely.

To investigate the effects of HES 200/0.5/6 on blood coagulation and platelet function we performed a randomised, controlled, cross-over study in healthy human subjects.

Materials and methods

The study was conducted according to the principles of the Helsinki Declaration of 1983 and approved by the institutional review board of the Academic Medical Centre, University of Amsterdam. Written, informed consent was obtained from all subjects.

Study design

The study was performed as a randomised, controlled, double-blind, cross-over experiment. Nine healthy men (20–39 years of age) participated in the study. Blood pressure, plasma creatinine, platelet count, prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured before the study and were normal. There was no history of increased bleeding tendency. Each subject was studied on two occasions with a 2–3 week washout period. Subjects were randomised to receive a 1h intravenous infusion of either 1000 ml 6% hydroxyethyl starch in NaCl 0.9% (mean MW 200.000, MS = 0.5, C2/C6 ratio = 6; HAES-steril 6%, Fresenius, 's-Hertogenbosch, the Netherlands) or 1000 ml albumin 4% in NaCl 0.9% as control experiment (200 ml Albumine 20% in NaCl 0.9%, Central Laboratory of the Red Cross Bloodtransfusion Service, Amsterdam, The Netherlands, with simultaneous infusion of 800 ml NaCl 0.9%). After the washout period the alternative infusion was administered.

Blood collection and bleeding time determination

Venous blood samples were obtained by separate venipunctures with the use of 21gauge butterfly needles, directly before the HES or albumin infusion and 30, 60, 120, 240 and 360 min thereafter. A bleeding time test was performed using a fully-automated incision-making device (Surgicutt, International Technidyne, Edison, New Jersey, USA), directly before and 60, 120 and 240 min after initiation of the infusion.

Assays

Routine haematological tests (platelet count, haemoglobin, haematocrit) were performed using standard laboratory methods. Platelet aggregation was measured at 37 °C with ristocetin (1.2 mg/ml, Paesi & Lorei, Hannan, Germany) and collagen (1 μg/ml, Chronolog, Havertown, Pa.) [4]. Light transmission of the stirred platelet-rich plasma was recorded relative to the platelet-poor plasma blank (Whole Blood Aggrometer, Chronolog, Havertown, Pa.). In vitro platelet function was also evaluated using the PFA-100 system (Dade Behring, Marburg, Germany) as described previously [5].

Coagulation times (aPTT and PT) were performed on a fully-automated coagulometer (Electra 1600C, Medical Automated, Pleasantville, N.Y.), using Actin FS and Thromborel S (Dade-Behring, Marburg, Germany), respectively. The functional assays of coagulation factors were performed by one-stage clotting assays with Recombiplastin (Ortho-Diagnostics Systems, Rariton, N.Y.) and Actin FS (Dade-Behring, Marburg, Germany). Antigenic levels of von Willebrand factor (vWF:ag) were measured using an ELISA. Ristocetin cofactor activity (vWF:RCo) was measured as described previously [6]. Prothrombin activation peptide (F1+2) and thrombin-antithrombin complexes (TATc) were performed with ELISAs (Dade-Behring, Marburg, Germany).

Plasma dilution

Plasma dilution by HES or albumin was calculated using the following equation: $D = Hb/Hb_0 \times (1\text{-}Ht_0)/(1\text{-}Ht)$ in which D is the dilution factor, Hb_0 and Ht_0 are the haemoglobin concentration and haematocrit before infusion and Hb and Ht are the haemoglobin and haematocrit after infusion [7]. The calculated concentration of a plasma substance after dilution is $C = C_0 \times D$, in which C_0 is the concentration before dilution, C is the concentration after dilution and D is the dilution factor.

Statistical analysis

Values are given as means ± SEM. Differences in results between the HES and albumin experiments were tested by repeated measurements analysis of variance. A p value less than 0.05 was considered to represent a significant difference.

Results

Bleeding time and platelet function

Baseline bleeding time was normal in all subjects. Infusion of both HES and albumin resulted in a slight prolongation of bleeding time at 60 min (from 3.7 ± 0.5 to 5.3 ± 0.6 min after HES and from 3.9 ± 0.6 to 4.9 ± 0.7 min after albumin). However, the difference between the HES and albumin experiments did not reach statistical significance ($p = 0.08$). The platelet count slightly decreased during infusion of either HES or albumin (–13 ± 2% vs –11 ± 2%, p = NS). Platelet aggregation induced by ristocetin or collagen was normal in all experiments and not influenced by either infusion (data not shown). Results of the platelet function analyser PFA-100 are shown in Fig. 1. Closure time with both collagen-epinephrine and collagen-ADP test cartridges was significantly increased after infusion of HES.

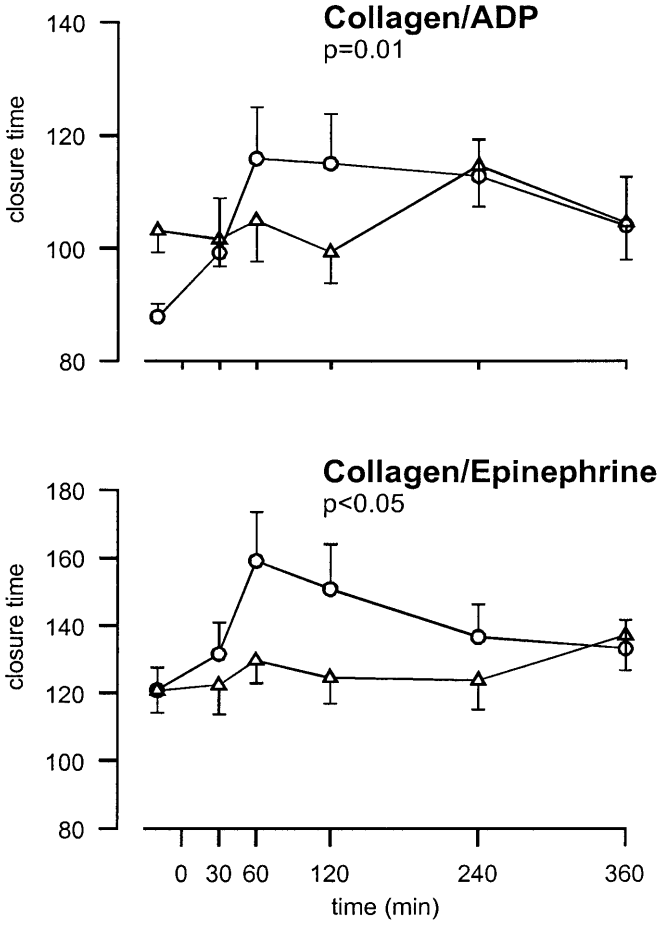

Fig. 1 In vitro platelet function measured with PFA-100 analyser. Mean ± SEM closure time was measured with collagen/ADP (**top**) and collagen/epinephrine cartridges (**bottom**) after intravenous infusion of HES *(circles)* or albumin *(triangles)*. Probability values represent difference between HES and albumin experiments by multiple measurements analysis of variance

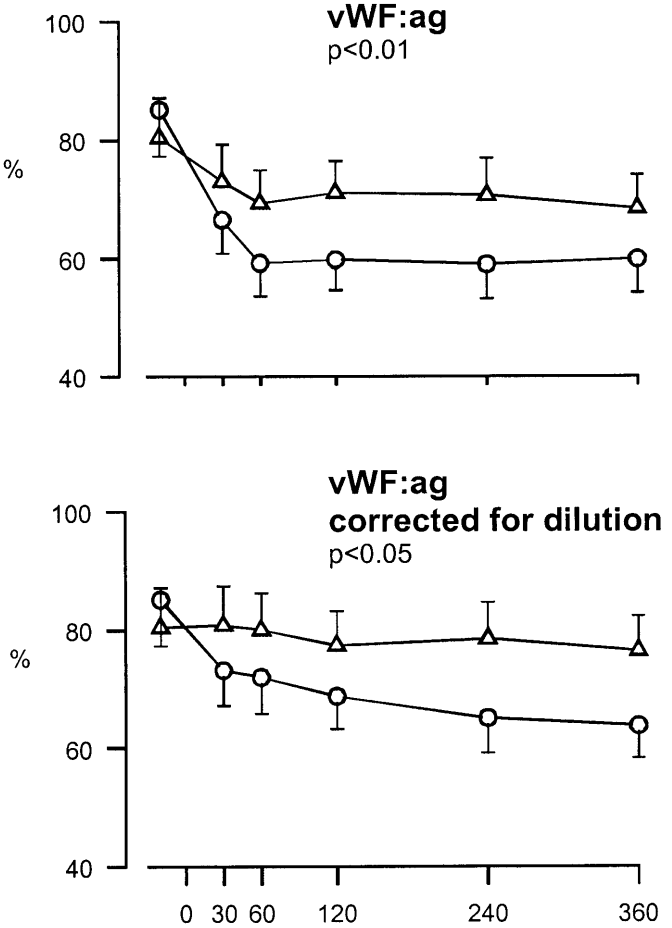

Fig. 2 (**Top**) Mean ± SEM plasma concentrations of vWF:ag after intravenous infusion of HES *(circles)* or albumin *(triangles)*. The **lower panel** shows vWF:ag after correction for dilution by multiplying by the dilution factor D. Probability values represent the differences between HES and albumin experiments by multiple measurements analysis of variance

Coagulation studies

Baseline values of PT, aPTT and functional assays of coagulation factors were similar in both study groups. In the HES group there was a trend to prolonged aPTT values, whereas these remained unchanged in the albumin group (3.2 s increase from baseline after HES vs 0.4 s after albumin, $p = 0.09$). No significant differences in PT, fibrinogen, factor V and factor VII values were found between the treatment groups (data not shown). Thrombin generation as measured by prothrombin activation peptide F1+2 and TATc (data not shown) was not influenced by either infusion.

Von Willebrand factor

The results of vWF:ag are presented in Fig. 2. Plasma vWF levels decreased in both groups. This decrease was more pronounced after HES compared to albumin (from 85 ± 8% at baseline to 59 ± 6% at 60 min after HES and from 80 ± 7% to 69 ± 8% after albumin, $p < 0.01$). In the albumin group, the decrease in vWF could be explained by dilution by the infused fluid, whereas in the HES group vWF decreased more than by dilution only ($p < 0.05$). Circulating levels of factor VIII:c decreased after HES from 83 ± 7% to 63 ± 7% and after albumin from 88 ± 6% to 74 ± 7% at 60 min ($p = 0.06$). Parallel to the decrease in antigenic levels of vWF, we also observed a decrease in ristocetin cofactor activity from 93 ± 4 to 67 ± 4% after HES and from 79 ± 5 to 75 ± 5 after albumin ($p < 0.01$).

Plasma dilution

No difference was observed in plasma dilution between the two study groups. Dilution factors at 30, 60, 120, 240 and 360 min were 0.89, 0.81, 0.84, 0.87 and 0.93 after HES infusion and 0.93, 0.87, 0.91, 0.89 and 0.91 after albumin (p = NS).

Discussion

In this study we found a HES-induced decrease of circulating levels of factor VIII:c and von Willebrand factor. After correction for dilution of plasma by the infused fluid, vWF:ag levels did not change in the albumin experiments, whereas a 26% decrease was found after infusion of HES. Associated with the reduction in vWF, we did find an impairment of in vitro platelet function as determined with the PFA-100 platelet analyser. Indeed, this PFA-100 analyser has been shown to be very sensitive and specific in detecting von Willebrand disease [5]. No significant differences were observed in bleeding time and platelet aggregation measurements, but the power of this study was insufficient to exclude such an effect. HES administration resulted in only a small prolongation of aPTT of borderline significance. This was probably caused by the decrease in factor VIII/vWF in combination with some dilution of coagulation factors. Thrombin generation as measured by prothrombin fragment F1+2 was unchanged after both infusions.

Reviewing the literature on the effects of HES on haemostasis yields conflicting results. High MW HES can prolong aPTT values, decrease factor VIII:c and vWF [1] and induce increased post-operative blood loss [8]. However, this effect of HES on haemostasis seems to depend on its in vivo MW [3]. Starch that is not substituted is degraded in vivo within minutes through the action of amylases in the blood. Hydroxyethylation of HES slows this process. Thus, in vivo MW is determined by the mean MW of the infused HES, its degree of substitution of hydroxyl by hydroxyethyl groups and its C2/C6 ratio. Accordingly, decreased vWF levels have been described after the administration of MMW-HES with a high degree of substitution [3], leading to in vivo accumulation of HES molecules with high MW. MMW-HES with a degree of substitution of 0.5 and a C2/C6 ratio of 6 (HES 200/0.5/6) is commonly used as a plasma substitute in Europe, is rapidly degradable without accumulation of macromolecules and is considered safe regarding coagulation disturbances. Treib et al. carried out a 10day haemodilution therapy in neurological patients and found no influence on factor VIII:c and vWF after HES 200/0.5/6 administration [3]. A potential explanation for the discrepancy between Treib's findings in patients and the decreased VIII:c and vWF levels in our study is the possibility that vWF, acting as an acute-phase protein, increases in acutely ill patients, masking the lowering effects of HES infusion.

Although statistically significant, the magnitude of the effects of HES in our study was only modest. It seems unlikely that HES, when given to patients in quantities comparable to the amount given in this study (i.e. 1 l), will induce an important impairment of haemostasis. This is in accordance with clinical studies that found no increased bleeding tendency after administration of MMW-HES [8, 9]. Nevertheless, especially when given in large quantities, HES could induce a clinically relevant bleeding tendency, in particular in patients with already low circulating levels of vWF or with other hereditary or acquired defects in haemostasis. Unfortunately, alternatives for HES have also been associated with disturbed haemostasis. Dextran infusion lowers factor VIII in plasma and prolongs the bleeding time [10]. Gelatin-based plasma substitutes induce a decrease of vWF, prolong bleeding time and decrease thrombin generation [6]. Albumin is expensive and its use on intensive care units has been associated with increased mortality [11].

Few studies have been published comparing the effects of different plasma substitutes on post-operative blood loss. One study found increased blood loss after orthopaedic surgery when HES 200/0.5/6 was given, compared to gelatin [12]. However, other studies found no difference in post-operative blood loss when MMW-HES was compared with gelatin [8, 9] or albumin [8]. Based on the present study and on a review of the literature, there is no reason to restrict the use of MMW-HES as a plasma substitute in most patients. However, in situations when the vWF lowering effects become clinically relevant (e.g. if massive amounts of plasma substitutes are given in bleeding patients or when other disturbances in haemostasis exist), the use of fresh frozen plasma or desmopressin (DDAVP) [2] to increase vWF levels should be considered.

We conclude that rapidly degradable MMW-HES 200/0.5/6 induces a mild reduction of plasma vWF, associated with impaired platelet function. Caution should be exercised when large amounts of HES are administered and when pre-existent disturbances in haemostasis exist.

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