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Effect of the carrier solution for hydroxyethyl starch on platelet aggregation and clot formation

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Editor's key points

- Hydroxyethyl starch colloid solutions can impair both platelet function and fibrinogen polymerization, but the contribution of the carrier solution is unclear.
- The effects of normal saline or balanced electrolyte carrier solutions on platelet aggregation and viscoelastic coagulation parameters were tested in healthy volunteers.
- The carrier solution itself had only small effects on platelet aggregation or clot formation in healthy subjects at the volumes infused.

Background. Hydroxyethyl starch (HES) solutions alter blood coagulation, mainly platelet function and fibrinogen polymerization. Haemostasis can also be impaired by dilutional-hyperchloraemic acidosis induced by the HES carrier solution. We hypothesized that a saline-based tetrastarch carrier solution impairs parameters of blood coagulation more than a balanced carrier solution.

Methods. The study was designed as a prospective, double-blinded, randomized, cross-over trial in healthy male volunteers. At intervals of at least 10 days, 13 subjects received 20 ml kg^{-1} of balanced or saline-based tetrastarch over 2 h. Blood was subjected to blood gas analysis, assessment of platelet function [with multiple electrode aggregometry (MEA)], and clot formation (with rotational thrombelastometry).

Results. Maximum aggregation in response to adenosine diphosphate (ADP) decreased after saline-based HES infusion, but not after balanced solution-based HES infusion. ADP-induced platelet aggregation was significantly lower after saline-based HES compared with baseline (21%; $P<0.025$) and compared with balanced solution-based HES (17%; $P<0.025$). There were no significant changes in platelet aggregation induced by thrombin receptor-activating peptide and in any parameter of rotational thrombelastometry. Chloride concentrations were significantly higher after saline-based HES compared with balanced solution-based HES.

Conclusions. The carrier solution for HES up to 20 ml kg^{-1} had little impact on platelet aggregation or clot formation as assessed by MEA and rotational thrombelastometry, respectively. Further clinical studies are required to verify this finding in patients and to correlate results of whole blood aggregometry and rotational thrombelastometry with perioperative bleeding and transfusion requirements.

Keywords: blood, coagulation; diagnostic techniques and procedures, platelet function test; diagnostic techniques and procedures, thrombelastography; hydroxylethyl starch; isotonic solutions

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Intravascular volume therapy with hydroxyethyl starch (HES) solutions has become an established approach to correct absolute or relative hypovolaemia.[1] Besides the desired volume efficacy, HES can alter blood coagulation, mainly platelet function and fibrinogen polymerization.[2–7] Depending on the status of the haemostatic system, colloid-dependent dilutional coagulopathy can be beneficial by mitigating hypercoagulability or harmful by aggravating hypocoagulability and bleeding.[8] The extent of these effects depends on the physicochemical characteristics of HES macromolecules and their metabolism.[8] HES of medium or low molecular weight and low molar substitution is rapidly degraded and carries the lowest risk of anticoagulant side-effects.[9]

Initially, HES was dissolved in isotonic saline, which can induce dilutional-hyperchloraemic acidosis in large volumes.[10] Given the importance of acid–base and electrolyte homeostasis for optimal function of coagulation factors,[11] haemostasis could be impaired by HES molecules, the carrier solution, or both. Hence, the latest development regarding HES preparations is rapidly degradable HES dissolved in buffered, balanced electrolyte solutions.

Only a few studies are available comparing the effects of HES in different carrier solutions on blood coagulation.[12–14] *In vitro* studies are of limited value due to well-known problems caused by artificial measurement conditions.[15] We hypothesized that a saline-based carrier solution impairs blood coagulation more compared with a balanced carrier solution for a rapidly degraded HES. To test this hypothesis, we analysed platelet function and clot formation in healthy volunteers before, during, and

after infusion of tetrastarch dissolved in two different carrier solutions.

Methods

Institutional review board approval was obtained before the study (registration no. 188/2007). The study was designed as a prospective, double-blinded, randomized, cross-over trial in 13 healthy male volunteers (performed May to November 2008). Inclusion criteria were male sex, age 18–65 yr, ASA classification I, and no bleeding history.[16] After written informed consent, potential subjects underwent physical examination and blood analysis for platelet count, prothrombin time, activated partial thromboplastin time, fibrinogen, creatinine and blood urea nitrogen concentration, and blood type. Coagulation time and fibrinogen concentration (Clauss method) were determined using an automated coagulation analyzer (STA-R Evolution, Stago, Asnieres, France). Exclusion criteria were BMI >25 kg m^{-2}, cardiac or renal insufficiency, known bleeding disorders, and/or blood type O. The latter were excluded because application of HES induces a more pronounced inhibition of blood coagulation in carriers of blood type 0.[17] Female volunteers were not considered because ovarian hormones have an impact on platelet function.[18 19] Subjects were not allowed to take any medication 14 days before and during the study period.

After enrolment, volunteers were randomized to receive two different HES preparations (20 ml kg^{-1}) administered over 2 h at intervals of at least 10 days in order to avoid a potential confounding effect of the first infusion on the second. One solution was a non-balanced potato-derived tetrastarch 6% 130/0.42 C2/C6 ratio 6:1 in isotonic saline (sodium 154 mmol litre^{-1}, chloride 154 mmol litre^{-1}) (Venofundin®, B. Braun, Maria Enzersdorf, Austria), and the other was a balanced potato-derived HES 6% 130/0.42 C2/C6 ratio 6:1 dissolved in Ringer's acetate (sodium 130 mmol litre^{-1}, chloride 112.5 mmol litre^{-1}, potassium 5.5 mmol litre^{-1}, calcium 1 mmol litre^{-1}, magnesium 1 mmol litre^{-1}, and acetate 27 mmol litre^{-1}) (Vitafusal®, Serumwerk Bernburg, Bernburg, Germany). Subjects and supervisors were blinded with regard to the test solution. Blood was drawn before infusion, after infusion of 10 ml kg^{-1} test solution (at 1 h), and at the end of the infusion (at 2 h). For each blood collection, atraumatic venipuncture was carried out with minimum stasis using a 21 G butterfly needle. The first 4 ml was always discarded, and samples were subjected to blood gas analysis immediately after withdrawal. Platelet function analysis and viscoelastic coagulation testing were performed within 2 h.[20 21]

Blood gas analysis

Blood was drawn into a Portex® (Portex, Keene, NH, USA) blood gas sample syringe. Venous blood gas analysis was performed using a standard analyzer (Radiometer Copenhagen®, Brønshøj, Denmark), and included determination of pH, base excess (BE), bicarbonate (HCO_3^-), haemoglobin, and electrolyte (sodium, chloride, and ionized calcium) concentrations.

Multiple electrode aggregometry

Blood was drawn into a 3.5 ml DTI-tube (direct thrombin inhibitor blood collection tube; Verum Diagnostica GmbH, Munich, Germany). Platelet function was determined implementing whole blood platelet aggregometry with Multiplate® (Verum Diagnostica GmbH) as described.[20] The impedance change due to platelet aggregation is transformed into arbitrary aggregation units (AU) by the system software and plotted against time. Platelet aggregation was determined in response to adenosine diphosphate (ADP, 6.5 μM) and thrombin receptor-activating peptide-6 (TRAP, 32 μM) using the commercially available test reagents, which permit the assessment of different pathways of platelet activation. Results shown are maximum platelet aggregation expressed in AU.

Rotational thrombelastometry

Blood was drawn into plastic tubes containing 3.8% trisodium citrate (Vacuette™ tubes Greiner, Kremsmünster, Austria; 9:1 v/v). Global haemostatic assessment was performed using rotational thrombelastometry (ROTEM®, TEM Innovations, Munich, Germany) as described.[21 22] At each time point, two commercially available ROTEM® tests were performed according to the manufacturer's recommendations: tissue factor is the coagulation activator in EXTEM® and ellagic acid activates coagulation in INTEM®. Clotting time (CT), clot formation time (CFT), angle alpha (α), maximum clot firmness (MCF), and lysis index were documented. All ROTEM analyses were performed for 60 min.

Statistics

A power analysis based on *in vitro* and *ex vivo* pilot experiments revealed that a sample size of 12 was required in order to detect a difference of 15% in ADP-induced platelet aggregation (β=0.90, α=0.05). Data were tested for normal distribution using the Kolmogorov–Smirnov test. Analysis of variance was used to assess the effect of the formulation and the amount of the test infusion and their interaction. Two-sided paired Student's *t*-test was used for *post hoc* comparisons between controls before test infusion and after 10 and 20 ml kg^{-1} of infusion and also between the two test solutions. The level of significance was adjusted according to the Bonferroni–Holm correction. Corrected *P*-values of <0.025 were considered statistically significant. Values are given as mean (SD).

Results

Baseline characteristics of the 13 volunteer subjects are summarized in Table 1.

Blood gas analysis

There was a statistically significant decrease in haemoglobin concentration after 20 ml kg^{-1} of both test infusions, with no differences between the balanced and saline-based solution (Table 2). Chloride concentrations increased significantly after 20 ml kg^{-1} of tetrastarch infusion and were significantly

higher after saline-based HES infusion compared with balanced HES infusion. pH, BE, and HCO_3^- changed in opposite directions after saline-based and balanced HES infusion resulting in higher levels after balanced compared with saline-based infusions. There were no significant changes in calcium concentrations between the two solutions (Table 2).

Multiple electrode aggregometry

Maximum aggregation in response to ADP decreased after saline-based HES infusion but not after HES in balanced solution (Fig. 1). The decrease in ADP-induced platelet aggregation reached statistical significance after 20 ml kg^{-1} of saline-based tetrastarch ($P<0.025$). Compared with baseline values, there was a 9% and 21% decrease in ADP-induced platelet aggregation after saline-based tetrastarch infusion of 10 and 20 ml kg^{-1}, respectively. ADP-induced platelet aggregation was significantly lower after 20 ml kg^{-1} of saline-based HES compared with balanced HES infusion with a difference of 17%.

Table 1 Characteristics of the study population. BMI, body mass index; BUN, blood urea nitrogen; INR, international normalised ratio; PT, prothrombin time; aPTT, activated partial thromboplastin time. Data are number with median (range) for age, or mean (SD)

n	13
Age (yr)	23 (21-30)
BMI (kg m^{-2})	22.0 (1.7)
Platelet count (g $litre^{-1}$)	248 (57)
Creatinine (mg dl^{-1})	1.03 (0.11)
BUN (mg dl^{-1})	14.4 (3.1)
INR	1.1 (0.1)
aPTT (s)	36.8 (2.0)
Fibrinogen (mg dl^{-1})	225.1 (33.3)
Test infusion volume (ml)	1426 (160)

There were no significant changes after test infusions and between the two solutions with respect to TRAP-induced platelet aggregation (Fig. 1). The two other multiple electrode aggregometry (MEA) parameters (velocity of aggregation, area under the aggregation curve) showed the same pattern as maximum aggregation (data not shown). Time until MEA analyses was comparable for both solutions.

Rotational thrombelastometry

There were no significant differences between saline-based and balanced HES infusions in any of the ROTEM® parameters tested (Table 3). EXTEM® CT increased significantly during saline-based HES infusion compared with baseline but did not change after exposure to HES in balanced solution. In contrast, INTEM® CT increased significantly after infusion of 20 ml kg^{-1} HES in balanced solution but not after saline-based HES.

The increase in EXTEM® CFT and INTEM® CFT and also the decrease in EXTEM® α and INTEM® α were statistically significant even after 10 ml kg^{-1} of both test infusions. The decrease in EXTEM® MCF and INTEM® MCF was also statistically significant after 10 ml kg^{-1} of both test infusions ($P<0.025$). No increased lysis index was observed before and after test infusions. Time until ROTEM® analyses was comparable for both solutions.

Discussion

This is the first *ex vivo* study of the effect of HES carrier solutions on coagulation parameters. The carrier solution of tetrastarch had minimal effects on platelet aggregation assessed by MEA and clot formation assessed by ROTEM® at the doses studied. There was no significant difference in viscoelastic parameters of ROTEM® analyses after test infusions of up to 20 ml kg^{-1} of tetrastarch dissolved in isotonic saline or balanced solution. In whole blood MEA using the strong platelet agonist TRAP, there were no differences after progressive haemodilution. The only significant signal

Table 2 Blood gas analyses before and after test infusions. Hb, haemoglobin; BE, base excess; BW, bodyweight. Data are mean (SD). $P<0.025$, *within test infusion group; #between test infusion groups

	Carrier solution	Control	After 10 ml kg^{-1} BW	After 20 ml kg^{-1} BW
Hb (g $litre^{-1}$)	Balanced	15.1 (0.8)	13.5 (0.7)	13.0 (0.5)*
	Saline-based	15.5 (0.8)	13.6 (0.8)	13.0 (0.8)*
pH	Balanced	7.35 (0.02)	7.36 (0.02)	7.37 (0.03)
	Saline-based	7.36 (0.04)	7.36 (0.04)	7.35 (0.02)#
BE (mmol $litre^{-1}$)	Balanced	1.39 (1.81)	2.02 (1.54)	2.27 (1.31)
	Saline-based	2.28 (1.34)	1.84 (1.64)	1.27 (1.39)#
HCO_3^- (mmol $litre^{-1}$)	Balanced	24.2 (1.19)	24.8 (1.09)	25.2 (1.18)
	Saline-based	24.9 (1.37)	24.7 (1.32)	24.2 (1.01)#
Cl^- (mmol $litre^{-1}$)	Balanced	105.2 (2.6)	105.7 (2.7)	106.3 (2.0)*
	Saline-based	104.8 (2.7)	107.5 (3.2)	107.8 (1.7)*,#
Ca^{2+} (mmol $litre^{-1}$)	Balanced	1.19 (0.12)	1.21 (0.06)	1.18 (0.08)
	Saline-based	1.22 (0.06)	1.19 (0.06)	1.19 (0.05)

was obtained in the MEA tests using the weak platelet agonist ADP. In this ADP test, platelet aggregability was lower after saline-based HES infusion. Platelet counts after 20 ml kg^{-1} test infusion remained above a methodological cut-off level of 100 G litre^{-1} [23] in our pilot experiments. The observed difference in ADP-induced MEA test results appears to represent a genuine effect of the saline carrier solution. Future studies correlating MEA values to clinical outcome are needed. Interestingly, in previous studies, blood loss and transfusion requirements were not different between patients exposed to balanced or saline-based colloid carrier solution.[14 24] These trials only assessed conventional coagulation tests, and showed no relevant differences between the solutions. These previous studies, however, did not use coagulation parameters such as platelet aggregation and overall clot strength that are suggested to be important in dilutional coagulopathy and clinical bleeding.[9–11] The present trial extends previous observations showing only negligible differences between balanced and non-balanced HES carrier solutions on platelet aggregation and clot strength.

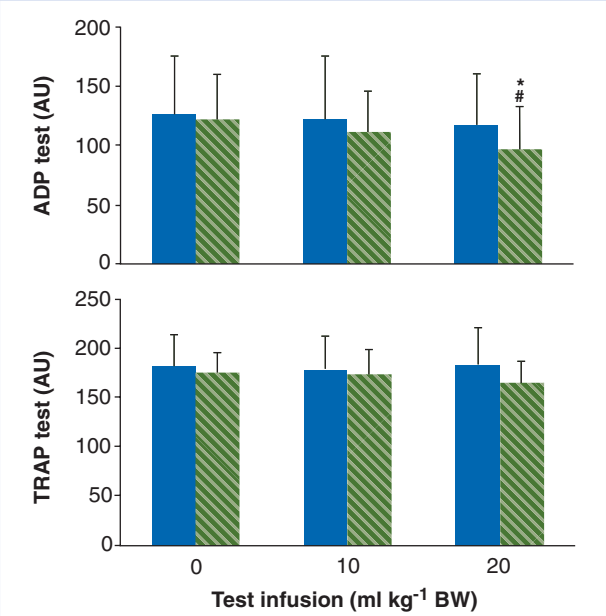

Fig 1 Platelet aggregation by MEA in response to ADP and TRAP after infusion of balanced tetrastarch (blue bars) or saline-based tetrastarch (green stripped bars). AU, aggregation units; ADP, adenosine diphosphate; MEA, multiple electrode aggregometry; TRAP, thrombin receptor-activating peptide. Data are mean (SD). $P<0.025$, *within test infusion group, #between test infusion groups.

Tetrastarch infusion can lead to dilutional coagulopathy. HES macromolecules can also have direct effects on haemostasis, with rapidly degradable HES such as tetrastarch having only minimal effects compared with slowly degradable HES such as penta-, hexa-, and hetastarch.[9] Haemostatic side-effects of tetrastarch were dose-dependent in the current study. MEA parameters showed a dose-dependent trend towards reduced platelet reactivity. Time to initial fibrin strand formation and the time and slope of clot formation assessed with ROTEM® were prolonged with increasing infusion volume. Also viscoelastic strength of the clot was weakened with increasing dose of tetrastarch. Reduced clot strength in the ROTEM® test with inhibition of the platelet contribution, the FIBTEM® test, has been interpreted as acquired fibrinogen deficiency[2 25] responsible for increasing blood loss. We did not measure FIBTEM® in this study, but it is important to consider that even after about 1.5 litre of tetrastarch infusion in our volunteers, the mean maximum clot strength of the more global EXTEM® and INTEM® tests remained well above trigger levels for therapeutic interventions defined experimentally in bleeding patients.[26 27] Several authors have investigated urgent reversal of impaired clot

Table 3 Results of EXTEM® and INTEM® tests. α, angle alpha; BW, bodyweight; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness. Data are mean (SD). $P<0.025$, *vs control within test infusion group

	Carrier solution	Control	After 10 ml kg^{-1}	After 20 ml kg^{-1}
EXTEM® CT (s)	Balanced	94.5 (34.8)	102.0 (28.7)	105.0 (28.9)
	Saline-based	91.0 (17.9)	115.5 (30.4)*	130.7 (35.7)*
EXTEM® CFT (s)	Balanced	146.3 (34.3)	185.4 (47.1)*	215.5 (50.5)*
	Saline-based	135.6 (27.7)	167.8 (36.3)*	196.2 (42.2)*
EXTEM® MCF (mm)	Balanced	53.5 (4.1)	47.8 (3.8)*	46.2 (5.2)*
	Saline-based	53.3 (5.2)	48.9 (3.9)*	46.3 (4.4)*
EXTEM® α (°)	Balanced	62.4 (5.0)	56.5 (5.7)*	54.1 (6.3)*
	Saline-based	63.9 (4.6)	59.5 (5.0)*	55.8 (5.0)*
INTEM® CT (s)	Balanced	196.9 (35.4)	220.3 (72.1)	247.5 (66.0)*
	Saline-based	209.2 (67.9)	217.9 (74.0)	232.5 (65.3)
INTEM® CFT (s)	Balanced	103.4 (29.7)	157.7 (57.4)*	179.9 (56.0)*
	Saline-based	110.4 (32.0)	143.1 (23.3)*	169.5 (28.9)*
INTEM® MCF (mm)	Balanced	57.9 (4.8)	51.2 (4.0)*	50.2 (4.1)*
	Saline-based	57.5 (4.0)	51.7 (3.0)*	50.6 (3.4)*
INTEM® α (°)	Balanced	72.0 (9.7)	61.8 (8.0)*	58.8.(7.8)*
	Saline-based	69.3 (5.3)	63.6 (3.7)*	59.5 (4.1)*

strength after colloidal haemodilution including administration of fibrinogen concentrate.[3 28] Our results suggest that infusion of up to 1.5 litre of tetrastarch does not lead to significant coagulopathy, as defined by ROTEM® parameters, requiring factor concentrate supplementation. However, factors confounding clot strength such as hypothermia or severe blood loss can require prohaemostatic therapy in surgical patients who also need colloidal fluid therapy of $<$1.5 litre.

Of note, we observed no increased fibrinolysis even after 20 ml kg^{-1} tetrastarch. *In vitro* studies show decreased clot stability in blood samples incubated with tissue plasminogen activator simulating hyperfibrinolytic conditions.[29 30] It appears that HES enhances the fibrinolytic response only in this *in vitro* lysis provocation test, but has no profibrinolytic effect *in vivo*.

In contrast to our previous study demonstrating an inhibitory effect of slowly degradable tetrastarch on TRAP-induced platelet function,[4] we observed no inhibition by rapidly degradable HES. In line with our previous studies demonstrating no inhibitory effect of rapidly degradable HES,[5] these findings imply that global platelet reactivity in response to the strong agonist thrombin remains preserved after tetrastarch but not after penta-, hexa-, and hetastarch infusion. We used ADP as a model of a weak platelet agonist in the present and previous studies, and observed inhibition of ADP-induced platelet aggregability after both saline-based hexastarch[4] and saline-based tetrastarch infusion (Fig. 1). The magnitude of inhibition by saline-based tetrastarch, however, was less compared with pharmacological inhibition by ADP-receptor antagonists as described for clopidogrel responders.[31] The underlying mechanism is unclear, but since extracellular coating is considered the pathomechanism of HES-dependent platelet dysfunction,[6] non-specific binding to the platelet surface might be reduced in a balanced buffered carrier milieu.

Homeostasis of calcium ions is relevant for coagulation enzyme function. Systemic levels of ionized calcium remained constant in our volunteers even after haemodilution with balanced tetrastarch containing calcium (Table 2). It appears that with infusion volumes up to 20 ml kg^{-1}, administration of calcium ions cannot be responsible for the observed differences between the saline-based and balanced carrier solution. The decrease in haemoglobin levels after HES infusion was similar (Table 2). Thus comparable volume efficacy cannot explain differences in ADP-induced platelet aggregation.

This study confirms a trend towards dilutional-hyperchloraemic acidosis by the use of saline-based carrier infusion. After infusion of saline-based starch, pH and BE decreased and chloride increased (Table 2). These changes were statistically significant but small. Even if the clinical relevance has been questioned,[9] it remains unknown if these additional derangements contribute to clinical symptoms in patients with severe acid–base disturbances.

This study has several limitations. First, only 20 ml kg^{-1} tetrastarch was administered; higher infusion volumes of up to the recommended maximum daily dose of 50 ml kg^{-1} might induce more pronounced effects. Secondly, in healthy volunteers without bleeding and fluid requirements, colloidal infusion results in hypervolaemic haemodilution which might alter endothelial response[32] and haemostasis. We chose a study design investigating volunteers because we wanted to isolate potential effects of tetrastarch carrier solutions (without confounding factors present in patients undergoing surgery). However, our findings in volunteers cannot directly be applied to patients in various clinical situations. Thirdly, with the test panel used, several haemostatic functions remain obscure. MEA and viscoelastic testing were used in the present study because they have been found to be associated with clinical outcome.[31 33] However, an increasing number of tests have become available for experimental and/or clinical use and permit visualization of aspects of the complex coagulation system. Among them, thrombin generation, platelet adhesion, secretion, and platelet procoagulant activity might contribute additional information in studies further investigating the anticoagulant effects of colloids. Fourthly, our results obtained after infusion of potato-derived tetrastarch in a carrier balanced with acetate cannot be extrapolated to tetrastarch dissolved in a carrier solution buffered with lactate or malate or to waxy maize-derived tetrastarch. We used potato-derived tetrastarch because at the time of the study this was the only balanced tetrastarch commercially available.

In conclusion, the carrier solution of HES infusion up to 20 ml kg^{-1} had only little impact on platelet aggregation assessed by MEA and clot formation assessed by ROTEM® in healthy volunteers. Further clinical studies are required to verify this finding in patients, after infusion of higher volumes up to the maximum daily doses, and to correlate results of MEA and ROTEM® with perioperative bleeding and transfusion requirements.

Declaration of interest

S.K.-L. received speaker's fees for lecturing and travel reimbursement from B. Braun, Fresenius Kabi, Verum Diagnostica, and TEM Innovations. G.S. received speaker's fees for lecturing and travel reimbursement from Fresenius Kabi, Verum Diagnostica, and TEM Innovations.

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