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Molecular weight of hydroxyethyl starch: is there an effect on blood coagulation and pharmacokinetics?‡

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Background. The development of hydroxyethyl starches (HES) with low impact on blood coagulation but higher volume effect compared with the currently used HES solutions is of clinical interest. We hypothesized that high molecular weight, low-substituted HES might possess these properties.

Methods. Thirty pigs were infused with three different HES solutions (20 ml kg^{-1}) with the same degree of molar substitution (0.42) but different molecular weights (130, 500 and 900 kDa). Serial blood samples were taken over 24 h and blood coagulation was assessed by Thromboelastograph® analysis and analysis of plasma coagulation. In addition, plasma concentration and *in vivo* molecular weight were determined and pharmacokinetic data were computed based on a two-compartment model.

Results. Thromboelastograph analysis and plasma coagulation tests did not reveal a more pronounced alteration of blood coagulation with HES 500 and HES 900 compared with HES 130. In contrast, HES 500 and HES 900 had a greater area under the plasma concentration–time curve [1542 (142) g min $litre^{-1}$, $P<0.001$, 1701 (321) g min $litre^{-1}$, $P<0.001$] than HES 130 [1156 (223) g min $litre^{-1}$] and alpha half life ($t^{1/2}_{\alpha}$) was longer for HES 500 [53.8 (8.6) min, $P<0.01$] and HES 900 [57.1 (12.3) min, $P<0.01$] than for HES 130 [39.9 (10.7) min]. Beta half life ($t^{1/2}_{\beta}$), however, was similar for all three types of HES [from 332 (100) to 381 (63) min].

Conclusions. In low-substituted HES, molecular weight is not a key factor in compromising blood coagulation. The longer initial intravascular persistence of high molecular weight low-substituted HES might result in a longer lasting volume effect.

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Hydroxyethyl starches (HES) are widely used plasma substitutes.[1 2] The clinical use of HES is limited by the effect on blood coagulation, associated with a reduction in circulating concentrations of factor VIII and von Willebrand factor (vWF) in a greater proportion than expected by simple plasma dilution.[3 4] Direct inhibition of platelet function through binding of HES to the platelet surface may also contribute to the compromising effects of HES on blood coagulation.[5]

HES is a modified branched natural polymer of amylopectin.[1] Besides its molecular weight, HES is characterized by its degree of hydroxyethylation, which is quantified by the molar substitution ratio. The molar substitution ratio is defined as the average number of hydroxyethyl groups per glucose residue present in the HES molecule.[1] Studies comparing high molecular weight *vs* low or medium molecular weight HES show a greater effect on blood coagulation of high molecular weight HES.[6 7] Thus, low and medium

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molecular, low-substituted HES preparations such as HES 130/0.4 (molecular weight 130 kDa, molar substitution 0.4) and HES 200/0.5 are preferentially used in clinical practice. The disadvantage of low or medium molecular HES preparations is shorter intravascular persistence, these products being more rapidly eliminated than high molecular weight HES.[3]

Molecular weight has been considered a key factor in determining blood coagulation effects of HES.[1] Nevertheless, the isolated effect of molecular weight on blood coagulation has not been systematically examined. To date, a reduction in molecular weight has always been associated with a reduction in molar substitution (from 450/0.7 to 200/0.5 to 130/0.4). In this study we therefore tested whether HES molecules with different molecular weight (130–900 kDa) but identical molar substitution (0.42) would affect blood coagulation differently. In addition, the intravascular retention of these HES molecules was assessed.

Materials and methods

The protocol was approved by the Veterinary Office of the Canton de Vaud, Switzerland. Pigs were handled according to the guidelines of the Swiss Federal Veterinary Office.

Animal preparation

On arrival at the laboratory, pigs weighing 38 (5) kg received intramuscular premedication with xylazine 2 mg kg^{-1}, ketamine 20 mg kg^{-1} and atropine 1 mg. Once sedated, anaesthesia was induced by administration of halothane 3% by mask, followed by tracheal intubation. Controlled ventilation was started with tidal volumes of 10 ml kg^{-1} and a ventilatory rate of 15 min^{-1} (Ventilator Draeger Sulla 909 V; Dräger, Luebeck, Germany). Anaesthesia was maintained with halothane 0.8–1.5% in oxygen and the internal jugular vein was catheterized. After infusion of HES and blood sampling via the internal jugular catheter during the initial 4 h after dosing, the catheter was removed, anaesthesia was terminated and the pig was subsequently transported back to the animal farm. The next morning, the pigs were brought back to the laboratory. Premedication and anaesthesia were administrated as described, and a catheter was placed into the contralateral internal jugular vein. After the final blood sample had been obtained, the animals were killed by an i.v. injection of pentobarbital 150 mg kg^{-1}.

HES characterization

HES solutions with different molecular weights but identical molar substitution were investigated. In order to ensure full identity of molar substitution and hydroxyethylation pattern, all three test solutions were manufactured using the same preparation. Thin-boiling waxy maize starch was suspended in water, activated by means of sodium hydroxide, and allowed to react with ethylene oxide for 2 h at 40°C. The amounts of waxy maize starch and ethylene oxide were chosen to yield HES with a molar substitution of 0.42. This original HES with its unique hydroxyethylation pattern was thereafter hydrolysed stepwise by means of treatment with hydrochloric acid to yield final HES solutions with molecular weights of 889 kDa (HES 900), 518 kDa (HES 500) and 122 kDa (HES 130). These were treated with activated carbon, purified by ultrafiltration, diluted to a final concentration of 6% (w/v) in 0.9% saline, added in volumes of 500 ml to glass bottles and heat-sterilized at 121°C for 20 min. For determination and verification of HES molecular weights, HES sample solutions were analysed in duplicate by GPC-MALLS (Wyatt Technology, Woldert, Germany) at a flow rate of 1 ml min^{-1} in phosphate buffer 70 mM, pH 7.0, using serial GPC columns HEMA Bio 40, 100 and 1000 (PSS, Mainz, Germany). Mean average molecular weight was calculated using Astra software (Wyatt Technology). The degree of molar substitution of the HES solutions was determined and verified in duplicate according to the method described by Hodges *et al.*[8] and Lee *et al.*[9] Physicochemical parameters are shown in Table 1.

Table 1 HES characterization

	HES 130/0.42	HES 500/0.42	HES 900/0.42
In vitro molecular weight (kDa)	122	518	889
Molar substitution	0.42	0.42	0.42
Concentration	6%	6%	6%
Viscosity (mPas) at shear rate of 128 s^{-1}	1.9	2.6	3.0

Experimental protocol

Animals were randomized into three groups of 10 pigs each, receiving HES 130, HES 500 or HES 900 at 20 ml kg^{-1} body weight of the respective 6% HES solution (blinded to the experimental investigators) was infused over 30 min as a top-load dose. Blood samples were taken before (baseline) and 5, 20, 40, 60, 120 and 240 min and 24 h after the end of the HES infusion.

Laboratory measurements

Whole blood measurements

Citrated blood samples at each sampling time were processed in the laboratory as follows.

Viscosity. One sample was immediately used for blood viscosity measurement (Rheostress® 1; Thermo Haake, Karlsruhe, Germany), for linear increasing shear rates from 1 to 240 s^{-1}. Viscosity at shear rates of 1, 4 and 128 s^{-1} were analysed.

Thromboelastograph analysis (TEG®; Haemoscope Corporation, Niles, IL, USA). This provides a more comprehensive way of monitoring the process of blood coagulation by testing the kinetics of the whole coagulation process.[10] It has been shown previously that TEG in conjunction with conventional coagulation assays provides a complementary

approach suitable for detection of coagulation abnormalities in the course of haemodilution by colloids.[11] The theory of TEG, including definition of measured parameters, has been extensively described earlier.[12 13]

Before TEG, blood samples were incubated for 1 h in a 37°C water bath.[14] Blood recalcification and TEG measurements were performed according to the manufacturer's instructions. The following TEG parameters are reported: reaction time (r), coagulation time (k), maximal amplitude (MA), angle (α) and coagulation index (CI).[14 15]

Plasma measurements

Blood samples were centrifuged at 3000 r.p.m. for 15 min at 4°C (Rotanta/RP; Hettich, Baech, Switzerland). Plasma viscosity measurements were performed as described above for blood viscosity measurements.

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were determined with an automated coagulation analyser (BCS; Dade Behring, Marburg, Germany) using a PT reagent containing recombinant tissue factor (Innovin®; Dade Behring) and an aPTT reagent containing ellagic acid (Actin FS®, Dade Behring), respectively. Functional activity of vWF was determined with a commercial ristocetin–cofactor assay (vWF RCA; Dade Behring) using an automated coagulation analyser (BCS, Dade Behring). Briefly, vWF activity was assessed by the ability to agglutinate fixed human platelets in the presence of ristocetin. Agglutination was measured turbidimetrically using the coagulation analyser. Antigenic vWF concentrations were assayed using a commercial ELISA kit (Asserachrom vWF antigenic; Roche Diagnostics, Rotkreuz, Switzerland) according to the manufacturer's instructions.

HES concentration was quantified after extraction from plasma and hydrolysis to glucose monomers. Briefly, plasma samples (1 ml) were incubated at 100°C for 60 min after addition of 0.5 ml KOH solution 35% (w/w) (Fluka, Buchs, Switzerland). HES was precipitated by adding ice-cold absolute ethanol (Fluka, Buchs, Switzerland) 10 ml to the supernatant of the reaction mixture and acid hydrolysed in 2 N HCl (Fluka) for 60 min at 100°C. Glucose determination was performed using an enzymatic test kit based on hexokinase/glucose 6-phosphatase (Boehringer Mannheim, Darmstadt, Germany). For determination of HES molecular weight, plasma proteins were eliminated by trichloroacetic acid precipitation [6.4% (w/w) end concentration] and neutralized supernatants were analysed by GPC-MALLS as described above (see HES characterization).

Pharmacokinetic modelling

Calculations of pharmacokinetic parameters were performed according to a two-compartment pharmacokinetic model with constant i.v. infusion rate and first-order output, using actual doses and duration of infusion. WinNonlin, Version 4.1 (Pharsight, Mountainview, CA, USA) was used for model-fitting using starting values automatically generated by the program and iterative reweighting with 1/(predicted). The quality of fit of the two-compartment model was confirmed by comparison of observed with predicted plasma concentrations according to the chosen two-compartment model. This comparison was completed by searching for absence of any systematic trend across the whole concentration range in the residual plot analysis.

Statistical analysis

Results are expressed as mean (SD). The two high molecular weight (500 and 900 kDa) HES solutions were compared with the low molecular weight (130 kDa) solution using the JMP 5.1 statistical package (SAS Institute, Cary, NC, USA). Data (with and without baseline correction) were analysed using a two-way ANOVA for repeated measures on one way (time) with Greenhouse–Geisser correction including solution and time effects. For statistical analysis of the pharmacokinetic parameters, unpaired Student's *t*-test was used. Bonferroni correction was used to avoid bias due to multiple comparisons when appropriate. $P<0.05$ was considered statistically significant.

Results

There were no significant differences among the three animal groups in body weight, baseline haemoglobin concentration and coagulation parameters.

Coagulation analyses

Plasma coagulation

All three HES solutions showed a significant time effect towards hypocoagulation for all plasma coagulation parameters (PT, aPTT, vWF activity and antigenic vWF concentrations), with a maximum effect immediately after infusion ($P<0.004$ for all parameters). On the basis of the baseline-corrected plasma coagulation parameters measured (PT, aPTT, vWF activity and antigenic vWF concentrations) there was no significant difference between HES 500 and HES 900 when compared with HES 130 (Fig. 1A–D).

TEG

The three HES solutions showed a significant time effect towards hypocoagulation for all TEG parameters after infusion of the respective solution ($P<0.002$ for all). The maximum effect was seen immediately after infusion. HES 500 and HES 900 did not impair blood coagulation more than HES 130 (Fig. 2A–E). Notably, the difference between MA in the HES 900 and the HES 130 group ($P=0.042$) (Fig. 2C) was not statistically significant, because of Bonferroni correction.

HES pharmacokinetics

The concentration curve of the HES preparations was adequately fitted by a two-compartment model, as shown

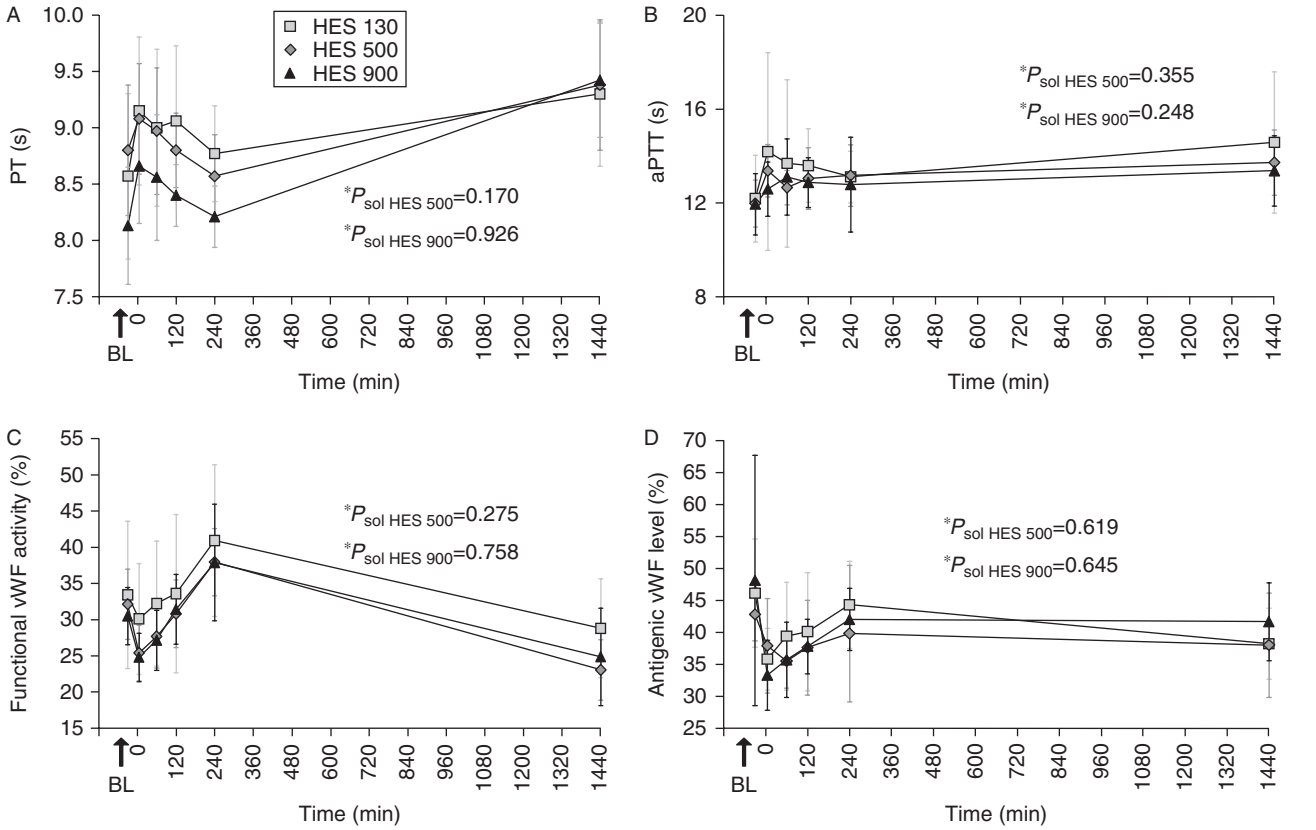

Fig 1 Time-course of plasma coagulation parameters: PT (A), aPTT (B), functional vWF activity (C) and antigenic vWF concentration (D). HES was infused over 30 min and PT, aPTT, vWF activity and antigenic vWF concentration was determined before [baseline (BL); arrow] and 5, 60, 120, 240 and 1440 min after the end of the infusion (0 min). Results are mean (SD). *Solution effect of HES 500 *vs* HES 130 ($P_{sol\ HES\ 500}$) and HES 900 *vs* HES 130 ($P_{sol\ HES\ 900}$) as determined by two-way ANOVA.

by the excellent correlation between observed *vs* predicted plasma concentrations (R^2=0.997) (Fig. 3A). This was confirmed by the absence of any systematic trend across the whole concentration range in the residual plot analysis (Fig. 3B). In accordance with these results, the two-compartment model was usually chosen for modelling of HES pharmacokinetics.[16]

HES concentration was significantly higher for HES 500 (P=0.013) and HES 900 (P=0.014) than for HES 130 (Fig. 4A). Also, *in vivo* plasma HES molecular weight was higher for HES 500 (P<0.001) and HES 900 (P<0.001) than for HES 130 (Fig. 4B).

The area under the plasma concentration–time curve was greater in the HES 500 group (P<0.001) and the HES 900 group (P<0.001) compared with the HES 130 group. Initial half-life $t_{\alpha}^{½}$ (HES 500, P=0.006; HES 900, P=0.006) was significantly higher for HES 500 and HES 900 compared with HES 130. In contrast, terminal half-life $t_{\beta}^{½}$ did not differ significantly between groups (Table 2).

Viscosity

Blood and plasma viscosity measurements showed for all three solutions and at any of the three shear rates analysed (1, 4 and 128 s^{-1}) a significant effect over time (P<0.05 for all). However, there were no significant differences in the HES 500 and HES 900 groups compared with the HES 130 group (Fig. 5A–F).

Discussion

This study indicates that low-substituted high molecular weight HES (HES 500/0.4, HES 900/0.4) does not compromise blood coagulation to a greater extent than low-substituted low molecular HES (130/0.4). In contrast, low-substituted high molecular HES results in higher plasma concentration and a longer intravascular persistence without elevated plasma concentration at 24 h.

This is a surprising finding since a high molecular weight has generally been considered a significant factor in determining the effect of a given HES solution on blood coagulation, in that the higher the molecular weight the more the blood coagulation is expected to be compromised.[16] However, the development of HES has so far been characterized by a concomitant reduction in molecular weight and molar substitution, from HES 450/0.7 to HES 200/0.5 to HES 130/0.4. We have now found that, at low substitution, high molecular weight HES does not

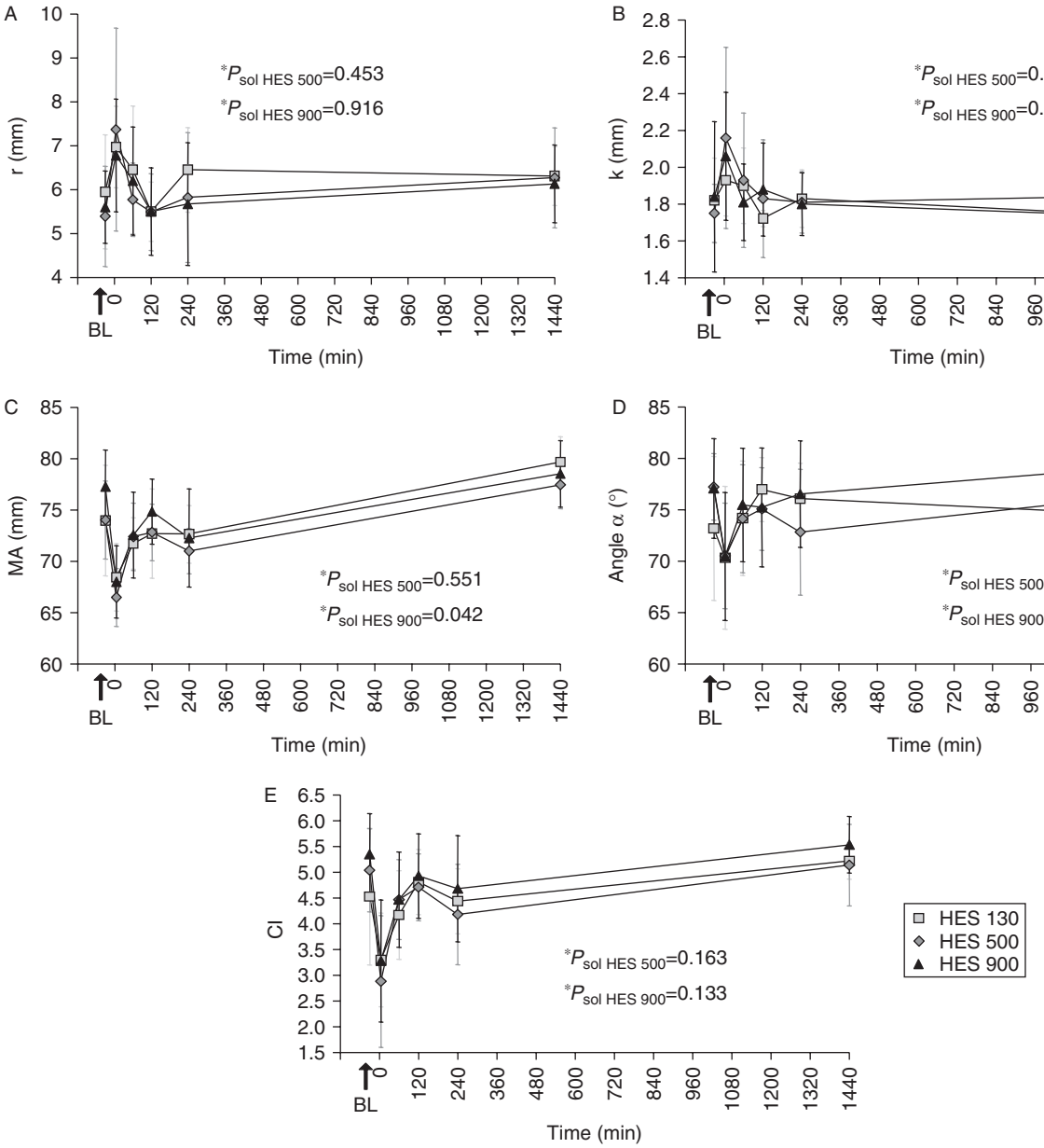

Fig 2 Time-course of the main TEG parameters: reaction time (r) (A), coagulation time (k) (B), maximal amplitude (MA) (C), angle (α) (D) and coagulation index (CI) (E). HES was infused over 30 min and TEG was determined before [baseline (BL); arrow] and 5, 60, 120, 240 and 1440 min after the end of the infusion (0 min). Results are mean (SD). *Solution effect of HES 500 *vs* HES 130 ($P_{sol\ HES\ 500}$) and HES 900 *vs* HES 130 ($P_{sol\ HES\ 900}$) as determined by two-way ANOVA.

compromise blood coagulation more than low molecular weight HES (Figs 1 and 2).

This is particularly remarkable for two reasons. First, the measured plasma concentrations of HES 500 and HES 900 were higher than the plasma concentration of HES 130 (Fig. 4A); secondly, the *in vivo* molecular weight of HES 500 and HES 900 was higher than the *in vivo* molecular weight of HES 130 (Fig. 4B). This is an important observation since *in vivo* molecular weight has been described as being more important in terms of effect on blood coagulation than *in vitro* molecular weight, the molecular weight of the HES molecules before infusion.[17 18] The differentiation between *in vitro* and *in vivo* molecular weight is clearly important, given the large difference regularly observed[18] and again found in this study (Fig. 4B and Table 1). Nevertheless, the effect on blood coagulation of low-substituted high molecular weight HES is not greater than that of low-substituted low molecular weight HES, indicating that molecular weight is not a key factor in determining the effect on blood coagulation.

The higher concentrations over time and the greater area under the curve (AUC) of the high molecular HES suggest a longer intravascular persistence of these solutions. The initial half-life $t_{\alpha}^{1/2}$ was longer with the high than with the low

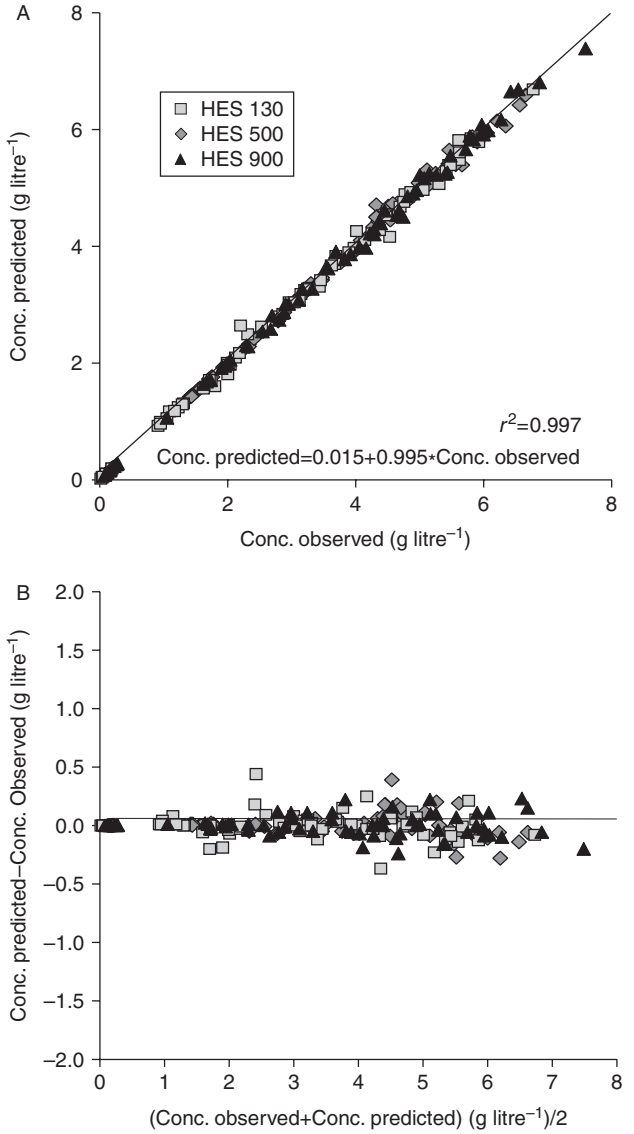

Fig 3 Pharmacokinetic modelling. The quality of fit between observed plasma concentration data and predicted data according to the chosen two-compartment model was analysed by linear regression analysis (A) and residual plot analysis (B). Conc.=concentration.

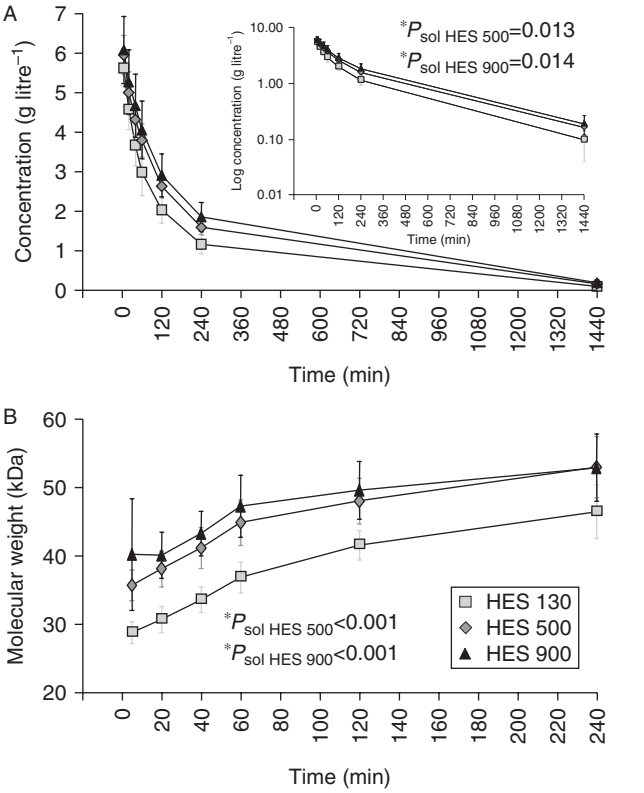

Fig 4 Time-course of serum HES concentration (A) and molecular weight (B). Insert in A shows the semi-logarithmic plot of concentration *vs* time. Results are mean (SD). *Solution effect of HES 500 *vs* HES 130 ($P_{sol\ HES\ 500}$) and HES 900 *vs* HES 130 ($P_{sol\ HES\ 900}$) as determined by two-way ANOVA.

Table 2 Pharmacokinetic parameters of HES 130, HES 500 and HES 900. Mean (SD). AUC, area under the curve; $t_{\alpha}^{½}$, initial half-life; $t_{\beta}^{½}$, terminal half-life. *P<0.01, **P<0.001

	HES 130	HES 500	HES 900
AUC (g min litre^{-1})	1156 (223)	1542 (142)**	1701 (321)**
$t_{\alpha}^{½}$ (min)	39.9 (10.7)	53.8 (8.6)*	57.1 (12.3)*
$t_{\beta}^{½}$ (min)	331.8 (100.0)	380.6 (63.3)	379.9 (75.8)

molecular weight HES. The difference in the initial half-life $t_{\alpha}^{½}$ may be explained by the fact that high molecular weight HES needs more time to be degraded to a level similar to that already present with low molecular weight HES from the beginning. The proximal part of the elimination kinetics may be important in terms of the effect as a volume replacement fluid, indicating a potentially higher intravascular volume effect of high molecular weight HES. In contrast to the initial half-life $t_{\alpha}^{½}$, terminal half-life $t_{\beta}^{½}$ did not show any difference between the groups. This suggests that there is no increased risk of accumulation after repeated-dose administration of low-substituted high molecular weight compared with low-substituted low molecular weight HES.

The porcine model is considered a suitable model for blood coagulation research.[19] It has been used to monitor coagulation changes under various interventions, such as in normovolaemic haemodilution, supracoeliac aortic cross-clamping and haemorrhagic shock.[20–22] More importantly, the changes in coagulation parameters due to HES infusions measured in our current animal study are similar to those observed in a previous study in humans.[11] In that study, low and medium molecular weight (70 and 200 kDa) HES were given to healthy human volunteers at a dose of 15 ml kg^{-1}, which is similar to the dose we used in our pig study (20 ml kg^{-1}). As indicated above, the changes in coagulation parameters (conventional coagulation tests and thromboelastography) over 24 h were strikingly similar in both human and porcine studies. In addition, it has been shown by McLoughlin *et al*. that the response of PT and aPTT to profound haemodilution is similar in humans and pigs.[20] Porcine coagulation thus seems to react quite

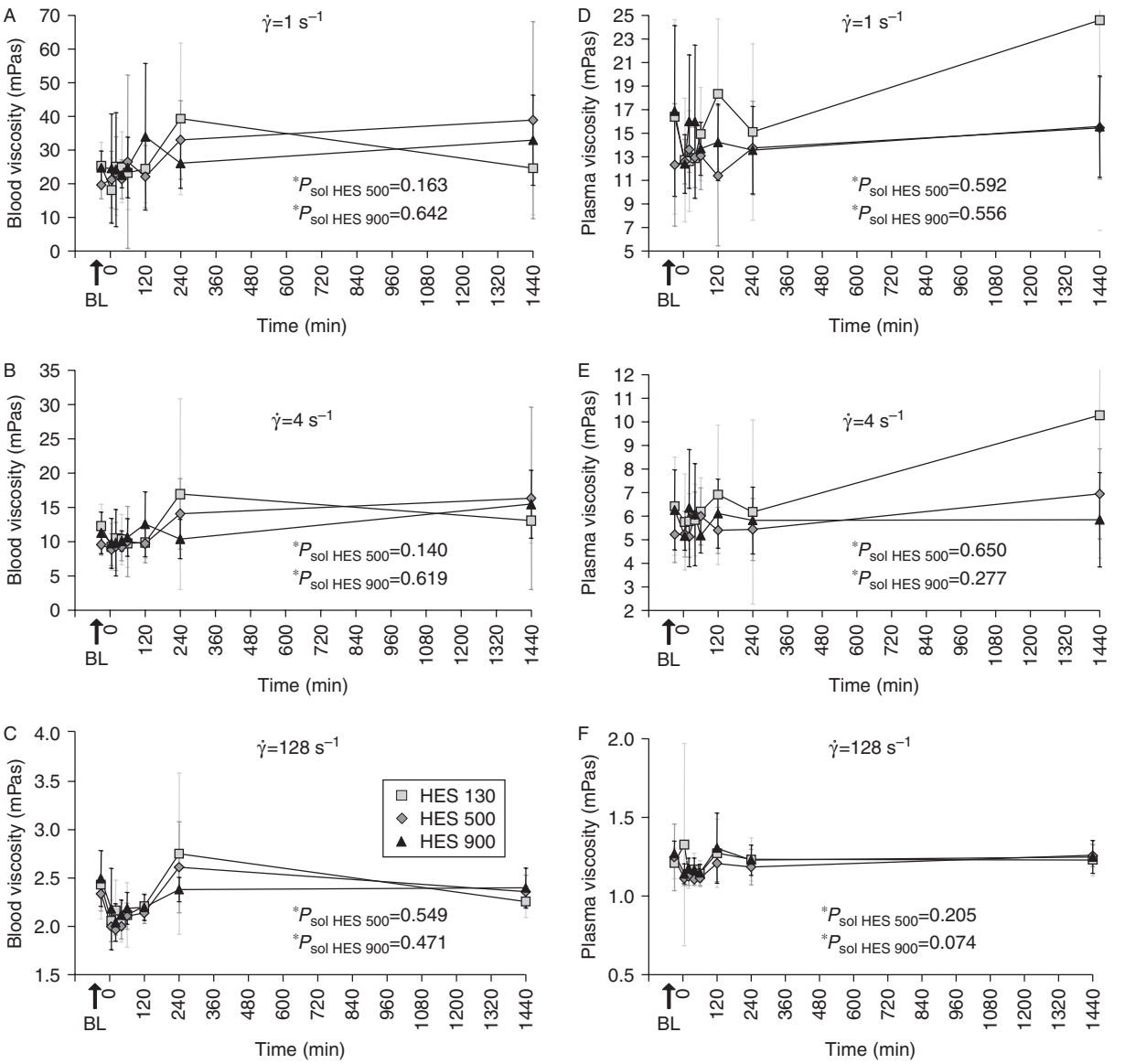

Fig 5 Blood (A–C) and plasma (D–F) viscosity measurements at shear rates ($\dot{\gamma}$) of 1 s^{-1} (A, D), 4 s^{-1} (B, E) and 128 s^{-1} (C, F). Results are mean (SD). *Solution effect of HES 500 *vs* HES 130 ($P_{sol\ HES\ 500}$) and HES 900 *vs* HES 130 ($P_{sol\ HES\ 900}$) as determined by two-way ANOVA. BL (arrow) denotes baseline.

similarly to HES infusion when compared with the human coagulation system.

The present study is limited to a 20 ml kg^{-1} top-load dose, resulting in haemodilution of approximately 20%. Future studies are necessary to assess whether low-substituted high molecular weight HES still does not compromise blood congulation more than low-substituted low molecular weight HES when higher doses are administered or when blood loss is compensated by the infusion of such HES solutions. In addition, redosing studies are necessary to confirm that low-substituted high molecular weight HES does not accumulate during prolonged dosing.

In summary, we have shown that low-substituted high molecular weight HES (HES 500/0.42 and HES 900/0.42) does not compromise blood coagulation to a greater extent than low-substituted low molecular weight HES (130/0.42) but has longer intravascular persistence. Molecular weight may not represent a key factor in determining the effects on blood coagulation of HES.

References

1 Treib J, Baron JF, Grauer MT, Strauss RG. An international view of hydroxyethyl starches. *Intensive Care Med* 1999; **25**: 258–68

2 Boldt J. New light on intravascular volume replacement regimens: what did we learn from the past three years? *Anesth Analg* 2003; **97**: 1595–604

3 Langeron O, Doelberg M, Ang ET, Bonnet F, Capdevila X, Coriat P. Voluven, a lower substituted novel hydroxyethyl starch

(HES 130/0.4), causes fewer effects on coagulation in major orthopedic surgery than HES 200/0.5. *Anesth Analg* 2001; **92**: 855–62

4 de Jonge E, Levi M. Effects of different plasma substitutes on blood coagulation: a comparative review. *Crit Care Med* 2001; **29**: 1261–7

5 Deusch E, Gamsjäger T, Kress HG, Kozek-Langenecker SA. Binding of hydroxyethyl starch molecules to the platelet surface. *Anesth Analg* 2003; **97**: 680–3

6 Strauss RG, Stansfield C, Henriksen RA, Villhauer PJ. Pentastarch may cause fewer effects on coagulation than hetastarch. *Transfusion* 1988; **28**: 257–60

7 Boldt J, Haisch G, Suttner S, Kumle B, Schellhaass A. Effects of a new modified, balanced hydroxyethyl starch preparation (Hextend) on measures of coagulation. *Br J Anaesth* 2002; **89**: 722–8

8 Hodges KL, Kester WE, Wiederrich DL, Grover JA. Determination of alkoxyl substitution in cellulose ethers by zeisel-gas chromatography. *Anal Chem* 1979; **51**: 2172–6

9 Lee YC, Baaske DM, Carter JE. Determination of the molar substitution ratio of hydroxyethyl starches by gas chromatography. *Anal Chem* 1983; **55**: 334–8

10 Pivalizza EG, Abramson DC. Thromboelastography: another point of view. *Anesth Analg* 2001; **93**: 517–8

11 Jamnicki M, Bombeli T, Seifert B, *et al*. Low- and medium-molecular-weight hydroxyethyl starches: comparison of their effect on blood coagulation. *Anesthesiology* 2000; **93**: 1231–7

12 Chandler WL. The thromboelastography and the thromboelastograph technique. *Semin Thromb Hemost* 1995; **21** Suppl 4: 1–6

13 Traverso CI, Caprini JA, Arcelus JI. The normal thromboelastogram and its interpretation. *Semin Thromb Hemost* 1995; **21** Suppl 4: 7–13

14 Camenzind V, Bombeli T, Seifert B, *et al*. Citrate storage affects Thrombelastograph analysis. *Anesthesiology* 2000; **92**: 1242–9

15 Whitten CW, Greilich PE. Thromboelastography: past, present, and future. *Anesthesiology* 2000; **92**: 1223–5

16 Waitzinger J, Bepperling F, Pabst G, Opitz J, Müller M, Baron JF. Pharmacokinetics and tolerability of a new hydroxyethyl starch (HES) specification [HES (130/0.4)] after single-dose infusion of 6% or 10% solutions in healthy volunteers. *Clin Drug Invest* 1998; **16**: 151–60

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

18 Treib J, Haass A, Pindur G. Coagulation disorders caused by hydroxyethyl starch. *Thromb Haemost* 1997; **78**: 974–83

19 Olsen AK, Hansen AK, Jespersen J, Marckmann P, Bladbjerg EM. The pig as a model in blood coagulation and fibrinolysis research. *Scand J Lab Anim Sci* 1999; **26**: 214–24

20 McLoughlin TM, Fontana JL, Alving B, Mongan PD, Bunger R. Profound normovolemic hemodilution: hemostatic effects in patients and in a porcine model. *Anesth Analg* 1996; **83**: 459–65

21 Anagnostopoulos PV, Shepard AD, Pipinos II, *et al*. Analysis of coagulation changes associated with supraceliac aortic crossclamping using thromboelastography. *J Surg Res* 2001; **98**: 52–8

22 Via D, Kaufmann C, Anderson D, Stanton K, Rhee P. Effect of hydroxyethyl starch on coagulopathy in a swine model of hemorrhagic shock resuscitation. *J Trauma* 2001; **50**: 1076–82