Changes in the molecular size distribution and post-transfusion survival of hydroxyethyl starch 350/0.60 as influenced by a lower degree of hydroxyethylation: a study in normal man

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SUMMARY The need to provide a greater rate of colloid clearance from blood than is presently available with the long-acting dosage form of HES 450/0.70 prompted the clinical investigation of a new species of hydroxyethyl starch (HES) possessing a M_w of 350 000 concomitant with a molar substitution of 0.60 (HES 350/0.60). The concentration of HES 350/0.60 in serum fell to half its peak value in 10.2 ± 0.7 (SD) hours (in contrast to an IT_{50} of approximately 25 hours with HES 450/0.70). Levels of glucose in serum remained elevated in normal fasted subjects after dosing, suggesting that catabolism of the infused HES 350/0.60 was occurring. Hydrolysis of residual HES 350/0.60 was confirmed by Sepharose CL-4B gel filtration analysis of material obtained from serum, showing continual production of *smaller* molecules relative to the injected solution (in contrast to HES 450/0.70, in which *intermediate* polymer fragments are recovered). Recovered HES 350/0.60 material displayed a K_{av} ranging between 0.74 and 0.72 and possessed a Stokes radius ($r = 45\text{\AA}$) similar to that of dextran 40 (M_w 41 000). HES 350/0.60 appears to offer the same advantages as the currently available long-acting HES 450/0.70 but is removed from blood approximately twice as rapidly. This more rapid hydrolysis of HES 350/0.60 may be useful, for example, in avoiding cumulative build-up of colloid in the blood of normal donors undergoing consecutive leucapheresis procedures.

A variety of synthetic colloids (eg, dextran and gelatin) have been utilised during centrifugal leucapheresis to enhance the overall efficiency of cell collection from normal donors.^{1 2} Clinical studies^{3 4} reported that a glycogen-like amylopectin (hydroxyethyl starch (HES)) derived from waxy starch and hydroxyethylated to reduce the rate of attack of alpha-amylase, was also useful in this regard. An initial study in man⁵ indicated that the intravascular clearance of this colloid, HES 450/0.70, possessing a weight average molecular weight (M_w) of 450 000 combined with a molar hydroxyethyl group substitution (MS)* of 0.70, was similar to dextran

(M_w 56 000-70 000) during the initial 24 hours after dosing. Recent reports,^{6 7} however, have demonstrated that even though the intravascular half-life (IT_{50}) of HES 450/0.70 ranges between 24 and 29 hours, residual colloid can be detected in blood for up to 17 weeks after injection. As the usefulness of HES 450/0.70 during leucapheresis would generally be restricted to a collection period of 2 to 4 hours, residual material remaining in blood after this period appears to serve no practical purpose and would certainly lead to increased plasma volumes in donors undergoing consecutive collection procedures.⁸ We have, therefore, investigated the plasma clearance of a new species of HES (HES 350/0.60) in which both the M_w and MS have been reduced. It was hoped that these physical alterations would offer the same advantages as HES 450/0.70, but clearance from blood could be enhanced.

*Average number of hydroxyethyl groups reacted per anhydroglucose residue. This may be calculated as: $MS = \frac{W_H}{1-W_H} \times \frac{162}{44}$, where W_H is the weight fraction of hydroxyethyl group in the polymer.

Material and methods

SUBJECTS AND PROCEDURES

Five normovolaemic men aged 20-21 possessing good health comprised the study group. Their body surface areas (BSA) ranged from 1.80 to 2.01 m², which corresponded approximately to whole blood volumes of 4500 to 5035 ml. The experiment was thoroughly explained to each subject, and their consent was obtained. Each subject had fasted for 12 hours before the injection of HES 350/0-60 and continued fasting up to and including the sample of blood taken 3 hours after dosing.

A standardised dose (30 g/m² BSA) of 6% (w/v) HES 350/0-60* suspended in 0.9% isotonic saline was infused through a 19-gauge cannula inserted in a forearm vein over a 52-67 minute period (average rate of injection was 0.24 ± 0.02 (SD) ml/kg per min). Samples of whole blood were collected before (baseline) and immediately after injection. Additional samples were taken 1, 3, 6, 12, 24, 48, and 192 hours after completion of the injection. Collected samples of whole blood were either dispensed into EDTA, Na₂ tubes for determination of the full blood count (FBC) or allowed to clot in a blood bank refrigerator maintained at 4 to 6°C. Serum was separated from the clotted sample within 45 minutes of taking by centrifugation at 2500 × g (Sorvall RC2-B centrifuge, USA) for 10 minutes at 4°C.

LABORATORY DETERMINATIONS

Total concentrations of carbohydrate in serum were assayed in triplicate by the anthrone method.⁹ The difference between the concentration of total carbohydrate and serum glucose (measured by the hexokinase method¹⁰) at each sampling interval constituted the concentration of HES 350/0-60. The FBC was determined in triplicate during each sampling by the Coulter Counter Model 'S' (Coulter Electronics, USA).

GEL FILTRATION

Trichloroacetic acid filtrates of serum collected at various intervals after dosing were concentrated by ultrafiltration and dialysed in Cellophane tubing (diameter 6.2 mm and pore size less than 7.2 nm). The resulting concentrate was applied to a column of Sepharose CL-4B (Pharmacia Fine Chemicals, Sweden) as previously described in detail.¹¹ A total of 50 mg of HES 350/0-60 (a volume of serum containing 10 mg of HES 350/0-60 from each of the five subjects) was pooled, thereby avoiding weighting the results according to concentration) was utilised

at each interval of sampling. The effluent solution was continuously monitored by a differential refractometer (Waters Model R4, USA) operating a chart recorder. The column was calibrated with Blue Dextran for void volume (V₀) and sodium iodide for total volume (V_t). The K_{av} for each of the eluted peaks was calculated according to the formula (where V_e = elution volume):

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}.$$

The Sepharose CL-4B column was further calibrated with Dextran 40 (M_w 41 000, M_n 26 000, M_w/M_n = 1.58) possessing a Stokes radius of 45 Å.¹²

Results

INTRAVASCULAR CONCENTRATION OF HES 350/0-60 AND GLUCOSE

In fasted normal subjects dosed with HES 350/0-60 the concentration of this colloid in blood diminished rapidly during the initial 6 hours after infusion (Fig. 1). Thereafter the disappearance took a more gradual course. The concentration of HES 350/0-60 in serum fell to half its peak value in approximately 10.2 ± 0.7 (SD) hours; 192 hours after dosing about 8% of the initial peak concentration (measured immediately after injection) remained in the blood. The concentration of glucose in serum rose after the period of infusion and was still elevated relative to baseline values 3 hours after injection.

MOLECULAR SIZE DISTRIBUTION OF RESIDUAL HES 350/0-60

Changes in the molecular size composition of circulating HES 350/0-60 determined at various intervals after dosing are shown in Figure 2. As can be seen, the injected solution is highly polydisperse and eluted with a K_{av} of 0.60. The HES 350/0-60 recovered from the intravascular space after injection, on the other hand, was of a distinctly narrower size distribution relative to the injected solution. In the interval 12 to 48 hours after injection, there is a sharpening of the distribution profile with signs of a return toward the low molecular weight region of the column. The calculated values of K_{av} (ranging between 0.74 and 0.72) for each peak of elution substantiated this observed movement of molecular size composition toward molecules of a smaller molecular weight. Polymer fragments of HES 350/0-60 remaining in blood after dosing possessed a Stokes radius (r = 45 Å) similar to that of Dextran 40, which eluted from the same column with K_{av} 0.76.

*M_w 350 000, intrinsic viscosity 0.20 dl/g, MS: 60 hydroxy-ethyl groups × 100 glucose residues.

Fig. 1 Concentration of HES 350/0-60 (■) and glucose (○) in serum of five normal men given (→) a standardised dose ($30 \text{ g/m}^2 \text{ BSA}$) of a 6% solution of HES 350/0-60. Each point represents the average of five determinations. The mean concentration of HES 350/0-60 was 10.85 ± 0.97 (SD) mg/ml immediately after injection. The intravascular concentration then progressively fell to 9.06 ± 0.80 , 7.69 ± 0.44 , 6.50 ± 0.41 , 4.95 ± 0.39 , 3.72 ± 0.21 , 2.54 ± 0.21 , and 0.86 ± 0.07 mg/ml 1, 3, 6, 12, 24, 48, and 192 hours after injection. The average concentration of glucose in serum (after a 12-hour fast) before dosing was 5.1 ± 0.4 (SD) mmol/l. This basal level rose to 7.1 ± 0.4 immediately after the period of infusion (53 ± 6 (SD) minutes) and then fell to 6.5 ± 0.6 and 6.3 ± 1.3 mmol/l 1 and 3 hours later. The concentrations of glucose after injection were corrected for haemodilution by changes which had occurred in the FBC.

Fig. 2 Changes in molecular size distribution of HES 350/0-60 after the dosing of five normal men with 943 ± 37 (SD) ml of a 6% solution. Gel filtration on a column of Sepharose CL-4B of 50 mg of HES 350/0-60 of M_w 350 000 and a similar quantity (pooled) of colloid recovered from serum immediately and 6, 12, 24, and 48 hours after injection. The infused HES 350/0-60 solution showed a peak with K_{av} of 0.60. Residual material recovered at various times up to 48 hours showed peaks with K_{av} ranging between 0.74 and 0.72. In general, the distribution of molecular size present in blood is less polydisperse relative to the injected solution with progressive sharpening of successive peaks. Recovered HES 350/0-60 polymer fragments had a Stokes radius ($r = 45 \text{ \AA}$)¹³ similar to that of Dextran 40 (which eluted from the same column of Sepharose CL-4B with K_{av} 0.76).

Discussion

Early clinical trials¹³ reported that HES 450/0-70, a

long-acting dosage form, was a valuable adjunct during centrifugal or intermittent leucapheresis, enhancing the overall collection of cells from both

normal subjects and patients with chronic granulocytic leukaemia. This glycogen-like colloid was also shown to be safe when administered to subjects on a repeated basis.¹³ However, recent reports^{8,14} have indicated that subjects receiving consecutive injections of HES 450/0-70 accumulate this colloid in blood with subsequent elevation of the plasma volume⁸ due to its H₂O-binding capability.

The period of usefulness of HES 450/0-70 during centrifugal or intermittent leucapheresis would be restricted to an interval of 2 to 4 hours during cell collection, and residual material remaining in blood after this time appears to serve no real purpose. By reducing both the M_w and MS of HES 450/0-70, we have demonstrated that a reasonable proportion of the initial peak dose of HES 350/0-60 remains 2 to 4 hours after injection (Fig. 1). More importantly, however, eight days after injection the intravascular concentration of HES 350/0-60 has fallen to 8%, as compared to 22-24% for HES 450/0-70 after seven days.^{6,7} We believe the reason for the greater rate of disappearance of HES 350/0-60 from blood may lie in both the pattern and amount of hydroxyethyl group substitution incorporated in the parent amylopectin molecule. Effective and sustained retardation of alpha-amylase attack implies that hydroxyethylation on any glucose residue within a specific five-unit amylose substrate deviates from acceptable patterns of substitution.¹⁵ For example, substitution on the 2-carbon of glucose-1, -3 or, -6 (glucose-1 is the reducing end) in the substrate unit would significantly hinder hydrolysis. It is also known¹⁶ that species of HES possessing a MS in the region of 0.7 to 2.0 have a greater frequency of di-, tri-, and tetra-substituted glucose residues, thus increasing the likelihood that unfavourable substitutions (and patterns of hydroxyethyl group attachment on glucose residues) will appear within the substrate unit, retarding catabolism. Extrapolation of these data suggest that a species of HES with a MS below 0.7 may contain a greater proportion of mono-substituted glucose residues, making attack by alpha-amylase more predictable.

This hypothesis is supported in the present investigation by the appearance of hyperglycaemia (indicating that catabolism of the injected colloid was occurring) and by the continual production of small molecular weight polymers relative to the injected solution (Fig. 2). Hyperglycaemia after dosing, concomitant with production of smaller molecular weight polymers, has also been confirmed clinically with an additional new species of HES having a MS of 0.43.¹⁷ On the other hand, serum concentrations of glucose are not usually elevated after dosing of normal man with HES 450/0-70,⁶ and intermediate (relative to the injected solution)

rather than smaller molecules are recovered from blood after injection.^{6,18} The recovery of intermediate size polymer fragments after dosing normal subjects with HES 450/0-70 is similar to what is observed after injecting man with dextran. As a function of time after dosing, polymer fragments of dextran remaining in blood become larger rather than smaller.¹⁹ The data obtained with HES 450/0-70 suggest that higher degrees of MS increase the frequency of multiple substitutions occurring on a single glucose residue, thus retarding to a greater extent attack mediated by alpha-amylase.⁶ Low degrees of MS (0.43-0.60) increase the likelihood that only mono-substitution will occur, allowing catabolism to proceed at a greater rate.

In conclusion, HES 350/0-60 offers the same advantages as HES 450/0-70 (HES 350/0-60 possesses the same critical molecular weight and critical concentration in regard to decreasing the suspension stability of whole blood²⁰) but is removed from the intravascular space approximately twice as rapidly. The more rapid hydrolysis of HES 350/0-60 would be advantageous to those donors undergoing multiple collection procedures with either the continuous- or intermittent-flow centrifuge.

Portions of this study were supported by the Alexander von Humboldt-Foundation, the SFB 68 of the Deutsche Forschungsgemeinschaft, and the Medical Research Council. Dr Mishler was an Alexander von Humboldt-Foundation senior research fellow.

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