Post-transfusion survival of hydroxyethyl starch 450/0.70 in man: a long-term study

JOHN MILTON MISHLER,¹ COLIN R RICKETTS,² AND EDWARD J PARKHOUSE²

From the ¹Medizinische Universitätsklinik Köln, D-5000 Köln 41, FRG, and the ²MRC Industrial Injuries and Burns Unit, Birmingham Accident Hospital, Birmingham B15 1NA, UK

SUMMARY Intravascular persistence concomitant with changes occurring in the circulating molecular size distribution were determined in five healthy normovolaemic men dosed with 7 ml/kg of a 6% (w/v) solution of a species of hydroxyethyl starch (HES 450/0.70) possessing a M_w of 450 000 daltons combined with a molar hydroxyethyl group substitution (MS) of 0.70 (70 hydroxyethyl groups/100 glucose residues). The concentration of HES 450/0.70 in serum fell to 24% of its peak value (measured 2 minutes post injection) one week after the infusion. By 17 weeks after injection, < 1.0% remained in the intravascular space. The HES 450/0.70 material recovered from the bloodstream 2 minutes after injection was shown by gel filtration on a column of Sepharose CL-4B to be less polydisperse than the injected solution. The K_{av} calculated for the peak of material eluted after one week showed a definite shift of molecular size toward that of a lower molecular weight composition. However, at four weeks the value of K_{av} indicated a shift toward the high molecular weight region of the injected solution, and by seven weeks this movement was quite pronounced. These data clearly indicate the complex nature of the removal of HES 450/0.70 from the intravascular space of man and appear to substantiate previous clinical studies reporting that the MS plays the major role influencing the rate of elimination of this material from the bloodstream.

Attachment of hydroxyethyl groups through ether linkages to the hydroxyl group of the parent amylopectin molecule can effectively retard the hydrolysis mediated by α -amylase.¹ The concept of adopting such a form of hydroxyethylated amylopectin as a potential colloidal agent to augment a diminished plasma volume was later proved feasible both experimentally² and clinically.³ From clinical studies conducted at a later date,⁴ a species of hydroxyethylated amylopectin (HES 450/0.70) possessing a weight average molecular weight (M_w) of 450 000 daltons combined with a molar hydroxyethyl group substitution (MS) of 0.70 (70 hydroxyethyl groups/100 glucose residues) was shown to persist in the bloodstream in a similar manner to a dextran having a M_w ranging between 56 000 and 70 000 daltons during the initial 24 hours after dosing. Even though HES 450/0.70 has been utilised clinically for over a decade in the management of hypovolaemia, little is known of its long-term persistence in man, and especially the nature of the changes occurring

in the circulating molecular size distribution of this material.

In some recent novel clinical uses of HES 450/0.70, such as an adjunct during centrifugal leucapheresis⁵ and as a cryoprotectant for platelets,⁶ infusion of this material serves no 'therapeutic purpose' other than to decrease the suspension stability of blood in normal donors in the former case, and as a cryoprotectant colloid in the latter application. In both these clinical situations information as to the elimination of HES 450/0.70 in normal man appears to be rather important. We have, therefore, endeavoured to document, during a long-term investigation in normal man, the persistence of HES 450/0.70 in blood concomitantly with determining the changes taking place in the molecular size composition of material remaining in the intravascular space.

Material and methods

SUBJECTS AND PROCEDURES

Five normovolaemic healthy men (aged 21 to 26)

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comprised the study group. Their body surface areas varied from 1.61 to 1.89 m², which corresponded approximately to whole blood volumes of 4025 to 4725 ml. The experiment was thoroughly explained to each subject and their consent was obtained.

A standardised dose (7 ml/kg) of a 6% (w/v) solution of HES 450/0.70 (M_w^- 450 000 daltons, number average molecular weight (M_n^-) 71 000 daltons, $M_w^-/M_n^- = 6.3$, intrinsic viscosity 0.25 dl/g, MS: 70 hydroxyethyl groups/100 glucose residues, Dr E Fresenius KG, FRG) in 0.9% isotonic saline was infused through a 19-gauge cannula inserted in a forearm vein over a 26- to 48-minute period (mean rate of injection was 0.20 ± 0.05 (SD) ml/kg/min). Samples of whole blood were collected into sterile plastic syringes (without anticoagulant) before (baseline) and 2 minutes after the injection. Samples were also taken 1, 2, 4, 7, 10, and 17 weeks after dosing. The samples of whole blood were allowed to clot in a blood bank refrigerator maintained at between 4 and 6°C. Serum was separated from the clotted sample within 45 minutes of collection by centrifugation at $2500 \times g$ (Sorvall RC2-B centrifuge, USA) for 10 minutes at 4°C.

LABORATORY DETERMINATIONS

After precipitation of the serum proteins with 5% (w/v) trichloroacetic acid (TCA), total concentration of carbohydrate was assayed in triplicate by the anthrone method.⁷ The difference between the concentration of total carbohydrate and serum glucose (measured by the hexokinase method⁸) at each sampling interval constituted the concentration of HES 450/0.70.

GEL FILTRATION

In determining the molecular size distribution of HES 450/0.70 remaining in the bloodstream after dosing, TCA filtrates of serum were concentrated by ultrafiltration and dialysed in Cellophane tubing (diameter 6.2 mm and a pore size of < 7.2 nm), and the resulting concentrate was applied to the gel filtration column. A total of 50 mg of HES 450/0.70 (a volume of serum containing 10 mg of HES 450/0.70 from each of the five subjects) was pooled, thereby avoiding weighting the results according to concentration) was used at each interval of sampling to provide sufficient material for analysis. Gel filtration was done on a column of Sepharose CL-4B (Pharmacia Fine Chemicals, Sweden) in M/15 sodium phosphate buffer (pH 7.5, 1 vol) plus 0.9% isotonic saline (9 vol), as previously described.⁹ The rate of elution ranged between 8.9 and 9.2 ml/h, and 5 ml fractions of elute were collected as required. The effluent solution was continuously monitored by a differential refractometer (Waters Model R4, USA)

operating a chart recorder. In some cases the material contained in each peak of eluted solution was ultrafiltrated, dialysed, and freeze-dried. The solid material so recovered was stained with Lugol's iodine solution in order to test for the presence of carbohydrate. Recorder reading was found to be linear with HES 450/0.70 concentration over a range of 0.1 mg/ml, corresponding with a 5.50 millivolt reading. All peaks eluted from the column were within this range of concentration. The column was calibrated with Blue Dextran for void volume (V_0) and sodium iodide for total volume (V_t). The K_{av} for each of the eluted peaks was calculated according to the formula (V_e = elution volume):

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

Results

INTRAVASCULAR CONCENTRATION OF HES 450/0.70

In five normal subjects dosed with an average of 449 ± 26 (SD) ml of a 6% solution of HES 450/0.70, the concentration of this material in the bloodstream fell to 24% of its peak value (measured 2 minutes after the termination of the infusion) one week after injection (Fig. 1). By 17 weeks after infusion $< 1.0\%$ remained in the intravascular space.

Fig. 1 Concentration of HES 450/0.70 in serum of five normal men dosed with 7 ml/kg of a 6% (w/v) solution of this material. Each point represents the mean of five determinations. The average concentration of HES 450/0.70 was 6.12 ± 0.51 (SD) mg/ml immediately (2 min) after injection. The intravascular concentration then fell progressively to 1.51 ± 0.16 , 0.96 ± 0.12 , 0.49 ± 0.13 , 0.22 ± 0.05 , and 0.15 ± 0.02 , mg/ml one, two, four, seven, and 10 weeks after the infusion. The concentration of HES 450/0.70 detected in the serum after 17 weeks was 0.06 ± 0.01 mg/ml, which represented $< 1.0\%$ of the peak concentration measured 2 min after injection.

CHANGES IN MOLECULAR COMPOSITION OF HES 450/0.70

Changes that had taken place in the molecular size distribution of circulating HES 450/0.70 determined at various intervals after dosing are shown in Figure 2. As can be readily observed, the molecular composition of the injected solution is made up of a wide distribution (polydispersion) of various molecular weight size material, as would be predicted by the ratio of M_w/M_n . However, 2 minutes after the infusion of HES 450/0.70 the recovered material is of a narrower size distribution relative to the injected solution. The K_{av} calculated for the peak of material eluted after one week showed a definite shift of molecular size toward that of a lower molecular weight composition (Table). However, four weeks after the infusion the value of K_{av} indicated a shift toward the high molecular weight region of the injected solution, and by seven weeks this movement was quite pronounced. The solid material recovered from the peaks of eluate obtained after four and seven weeks stained a reddish-brown colour with Lugol's iodine solution in the same manner as the HES 450/0.70 contained in the injected solution, confirming the presence of carbohydrate in these peaks.

There was no significant difference between the infrared spectrum of HES 450/0.70 recovered from

K_{av} values for elution peaks* obtained after separation of various molecular weight HES 450/0.70 material on a column of Sepharose CL-4B (see Material and methods).

	Injected HES 450/0.70 solution	Immediately after injection	Weeks after injection		
			1	4	7
K_{av}	0.57	0.67	0.70	0.68	0.60

*The peaks were obtained from samples of pooled serum and correspond to the elution profiles in Figure 2.

the infused solution and the spectra of material recovered from the plasma four and seven weeks after dosing.

Discussion

Amylopectin should be acceptable as a therapeutic colloid when infused into man, based on its similarity to normal body glycogen. Ordinary solutions of starch, however, are physically unstable and are rapidly catabolised in the blood of rabbits¹⁰ and canines¹¹ after intravenous dosing. On the other hand, physically stable branched polymers of starch, such as amylopectin, can be reacted with ethylene oxide in an alkaline environment,¹ enabling hydroxyethyl groups to be attached by ether linkages to the hydroxyl group of the glucose residue. Hydroxyethylation may attach the hydroxyethyl group on either the 2-, 3-, or 6-carbon of the glucose residue, thus effectively retarding the hydrolysis mediated by α -amylase contained in blood.^{12,13} In the clinically available species of HES, approximately 80% of the substitution of the hydroxyethyl group would be expected to take place on the 2-carbon.¹⁴ The predominant substitution of the hydroxyethyl group on the 2-carbon appears constant up to an MS of 1.4. This 2-carbon hydroxyl group is more acidic relative to those at either the 3-carbon or 6-carbon positions. The difference in acidity is caused by the electronic effects of the oxygen atoms.¹⁴ Another factor in this selective substitution on the 2-carbon is the possibility of stabilisation of the negative alkoxyion at either the 2-carbon or 3-carbon position by the proton of the adjacent hydroxyl group.

Of interest clinically is the observation that the actual position of the hydroxyethyl group, whether it be on the 2-, 3-, or 6-carbon of the glucose residue, appears to exert a significant influence on retardation of α -amylase catabolism.¹³ A diminished rate of hydrolysis by α -amylase is reported with substitution on the 2-carbon, or with multiple hydroxyethylations on the same carbon. However, less resistance to hydrolysis is reported to occur when the 2-carbon/6-

Fig. 2 Changes in molecular composition of HES 450/0.70 after the dosing of five normal men with 7 ml/kg of a 6% (w/v) solution. Gel filtration on a column of Sepharose CL-4B of 50 mg of HES 450/0.70 of M_w 450 000 daltons (—) and a similar quantity (pooled) of HES 450/0.70 recovered from serum 2 min after injection (---) and one (■—■), four (▲—▲), and seven (●—●) weeks after dosing. In general, the distribution of molecular size present in the blood-stream is less polydispersed than was observed in the injected material. After one week there is a definite shift of molecular size towards that of a lower molecular weight (LMW) composition. However, at four weeks there are signs of a shift toward the high molecular weight (HMW) region of the injected solution, and by seven weeks this movement is quite pronounced. The calculated value for the K_{av} of each eluted peak is given in the Table.

carbon substitution ratio is below 2.4. Effective retardation of α -amylase attack implies that hydroxyethylation on any glucose residue within a specific five-unit amylose substrate must deviate from the acceptable pattern of substitution.¹⁵ For example, substitution on the 2-carbon of glucose-1, -3, or -5 in the five-unit amylose substrate (reducing end is glucose-1) would significantly hinder attack by α -amylase.

In canine¹¹ or rabbit¹² models, the intravascular half-lives (IT_{50}) of various injected species of HES can be prolonged at any given M_w by increasing the MS, or, conversely, at any given MS increasing the M_w will impede clearance of this material from the circulation. In these studies, the MS was below 1.4, indicating that the substitution of the hydroxyethyl group took place in the majority of instances on the 2-carbon. In man, the actual persistence of any species of HES does not depend wholly on the relative molecular weight size distribution (M_w/M_n) of the injected solution but rather on the degree of MS.^{4,16-18} The clearance from the intravascular space during the initial hour after injection does depend on the M_w (more accurately on a subpopulation of molecules characterised by the M_n), but after this initial phase of rapid elimination the MS plays the major role, influencing catabolism and subsequent excretion through the kidneys. For example, given as a single bolus injection, the clinical IT_{50} s of HES 350/0.60,¹⁹ HES 264/0.43,¹⁸ and HES 150/0.70¹⁷ are approximately 12, 3, and 10 hours, respectively. In normal man, dosed on a single occasion with HES 450/0.70, the IT_{50} was reported to range between 24 and 28 hours.^{4,16,20} Up to 72 hours after a single intravenous dose the disappearance of HES 450/0.70 from the bloodstream can be described mathematically and appears to consist of three phases of elimination (rapid, intermediate, and slow IT_{50} s).⁴ Mathematically speaking, HES 450/0.70 is eliminated from the blood in a similar fashion for up to 24 hours each day after the infusion of this material on three consecutive days.²¹ However, the actual long-term persistence of HES 450/0.70 in blood appears to differ significantly from predictions made by mathematical models describing elimination over a relatively short period of observation.²⁰ In normal subjects injected with 60 g on a single occasion⁴ or 30 g on each of three consecutive days (total 90 g),²¹ the 1.0% level of remaining HES 450/0.70 material in the blood was mathematically predicted to occur 2.3 and 4.5 weeks after dosing, respectively. In a previous long-term study,²⁰ the 1.0% concentration of HES 450/0.70 in serum was not achieved even after 17 weeks. However, in the present study the concentration of HES 450/0.70 in serum fell to less than 1.0% 17 weeks after

injection (Fig. 1). The discrepancy between the disappearance of HES 450/0.70 from blood as predicted mathematically and what is actually observed may reside in the character of the residual material remaining after the rapid and intermediate phases of elimination. If the injected solution of HES 450/0.70 contains molecules in a small fractionated range combined with a homogenous pattern of hydroxyethylation, one assumes that disappearance could be predicted adequately by an equation of the first order. However, up to 72 hours after injection elimination is predicted only by an exponential function.^{4,21} These data clearly indicate the complex nature of the clearance of this material from the circulation.

The interplay between M_w (M_n) and MS during the slower phase(s) of elimination has been characterised by investigating the changes taking place in the molecular weight size composition of the residual material. In rabbits infused once with either HES 150/0.70 or HES 450/0.70, the Sepharose CL-4B elution profile of residual material recovered 24 hours after injection indicated the presence of an intermediate fraction occupying a position between the low and high molecular weight regions of the injected solution.⁹ In man, a similar intermediate fraction is recovered from blood three weeks after the third and final consecutive injection of HES 450/0.70.²² In the present study, a definite intermediate fraction was observed seven weeks after a single dose of this material was administered (Fig. 2; Table). However, in normal man infused once with a species of HES (HES 264/0.43) in which the MS was reduced 40%, only smaller molecular weight size material relative to the injected solution was recovered.²³ The appearance of an intermediate fraction after dosing with HES 450/0.70 may imply that a condition of steady-state hydrolysis had occurred in which the rate of catabolism of the low and high molecular weight fractions contained in the injected solution are similar. On the other hand, the continual movement of the molecular weight size distribution toward the low molecular weight region of the injected HES 264/0.43 solution suggests that catabolism of the higher molecular weight fraction contained in the injected solution may occur at a rate greater than the degradation of the low molecular weight fraction. It is known that as the MS approaches 1.0, the number of di-, tri-, and tetra-substituted residues of glucose becomes greater,¹⁴ thus increasing the likelihood that unfavourable patterns of substitution will be incorporated into any five-unit amylose substrate.¹⁵ The data contained in the present study and that previously published suggest that higher degrees of MS increase the number of multiple substitutions, thus retarding

to a greater extent the attack mediated by α -amylase. Low degrees of MS may increase the likelihood that only mono-substitution will occur, making attack by α -amylase more predictable.

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Requests for reprints to: Dr John Milton Mishler, IV, Medizinische Universitätsklinik Köln, Joseph-Stelzmann-Strasse 9, D-5000 Köln 41, FRG.