CHANGES IN THE MOLECULAR COMPOSITION OF CIRCULATING HYDROXYETHYL STARCH FOLLOWING CONSECUTIVE DAILY INFUSIONS IN MAN

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1 Changes in the circulating molecular composition of hydroxyethyl starch (HES) were determined in four normal men following three consecutive daily 500 ml infusions (total 1,500 ml), by passage of trichloroacetic acid filtrates of plasma through a Sepharose CL4B gel filtration column.

2 The HES recovered from the intravascular space 10 min following the injection on Days 1, 2 and 3, was of a narrower molecular size distribution than the injected material, with a noticeable shift to molecules of a low molecular weight (LMW) size.

3 The HES in the sampled plasma 24 h post-injection on Days 1, 2 and 3 consisted of molecules possessing a LMW distribution, concomitantly with a slight shift to molecules of a larger molecular size.

4 The HES recovered from the bloodstream 480 h after the third and final injection consisted of molecules possessing an intermediate size distribution, between LMW and high molecular weight (HMW) size material.

5 The results indicate that large HES molecules contained in the injected material are eliminated from the bloodstream; the HMW fraction at least partially by α -amylase mediated catabolism, and the resulting LMW fraction by excretion.

Introduction

Hydroxyethyl starch (HES), a high molecular weight volaemic colloid, has been efficaciously used in the clinical treatment of hypovolaemic shock (Gollub, Schechter, Hirose & Bailey, 1969; Solanke, 1968). In this regard, numerous studies (Metcalf, Papadopoulos, Tufaro & Barth, 1970; Mishler, Borberg, Emerson & Gross, 1977a, b; Solanke, 1968) have determined the intravascular clearance and renal excretion of this material in man, after single and multiple infusions. Even though these studies have endeavoured to measure the concentration of HES in plasma and urine, no information is available in man, as to the alterations in the molecular composition of circulating HES following single or repeated injections. In animals administered large volume infusions of HES, the molecular composition of this material in the intravascular space (Farrow, Hall & Ricketts, 1970) and in the excreted urine (Bogan, Gale & Walton, 1969), is of a narrower size distribution than that of the original injected material. The narrower size distribution in plasma following injection of the HES, is presumably due to smaller molecules being removed by filtration at the glomerulus, with large molecules being taken up by

cells of the reticuloendothelial system or catabolised through the action of α -amylase. In man, the question of changes in the molecular size composition of HES following single, and more importantly repeated injections, remains to be investigated.

Our purpose, therefore, was to determine the changes in the molecular composition of circulating HES in normal man, following three consecutive daily infusions.

Methods

Four normal men (ages 20–29 years) comprised the study group. Details of body surface area and blood volumes have been previously described (Mishler *et al.*, 1977b).

Five hundred millilitres of a 6% HES solution (M_w 450,000 daltons, M_n 70,000 daltons, intrinsic viscosity 0.25 ml g^{-1} , 6–7 hydroxyethyl groups/10 glucose units, McGaw Laboratories, Irvine, California, U.S.A., Lot No. A5N177A) in 0.9% sodium chloride solution, was infused through a

Figure 1 Changes in the molecular composition of HES recovered from human plasma, 10 min (a, upper graph) and 24 h (b, lower graph) post-injection on Days 1 (.....), 2 (-----), and 3 (—). The molecular size distribution of the original injected material (—) is shown for comparison. Gel filtration on a column of Sepharose CL4B with monitoring of the effluent by refractive index (further details in text). As reference points, the void volume (V_0) and total volume (V_i) are given. The distribution of molecular size present in the circulation, 10 min and 24 h post-injection on all days, is narrower than was present in the HES solution injected, and displays a noticeable shift to molecules of a LMW size.

19-gauge butterfly needle inserted in a forearm vein, on each of three consecutive days (total 1,500 ml). The infusion period ranged from 24–47 min (mean injection rate: $0.19 \text{ ml kg}^{-1} \text{ min}^{-1}$) on these twelve occasions. Blood samples with heparin anticoagulant were taken 10 min and 24 h following each injection, and the plasma separated. A further blood sample was taken 480 h after the third and final injection. Following precipitation of the plasma proteins with trichloroacetic acid, total carbohydrate concentrations were determined by the anthrone method (Brake, 1966). The difference between total carbohydrate and plasma glucose (measured by the d-glucose oxidase method) concentrations at each sampling, constituted the HES concentration. In determining the molecular size distribution of HES remaining in the intravascular space, the trichloroacetic acid filtrate was concentrated by dialysis in Cellophane tubing

(diameter: 6.2 mm; pore size: $<7.2 \text{ nm}$) and the concentrate applied to the gel filtration column. Plasma (1 ml) from each of the four subjects was pooled at each sampling interval, to provide sufficient material for analysis.

Gel filtration was done on a column of Sepharose CL4B in M/15 sodium phosphate buffer (pH 7.5, 1 volume) plus 0.9% sodium chloride solution (9 volumes) as previously described (Farrow *et al.*, 1970). The elution rate was $11\text{--}12 \text{ ml h}^{-1}$ and 5 ml fractions were collected as required. The effluent solution was monitored by a differential refractometer (Waters Model R4) operating a chart recorder. Experiments with solutions of known concentration revealed that the recorder readings were linear with concentration. The column was calibrated with Blue Dextran for void volume (V_0) and sodium iodide for total volume (V_i).

Figure 2 Gel filtration on a column of Sepharose CL4B of the original infused material (—), and HES recovered from human plasma, 24 (---) and 480 h (····) following the third and final injection. The HES recovered from the intravascular space 480 h post-injection consists of molecules having an intermediate size distribution, between LMW and HMW size material.

Results

Changes in the molecular size distribution of circulating HES, 10 min and 24 h following single and repeated injections, are shown in Figure 1. As can be seen, the molecular composition of the injected solution is made up of a wide distribution of varying molecular weight size material. The HES recovered from the intravascular space 10 min post-injection on all 3 days, however, was of a narrower size distribution, with a preponderance of LMW molecules. The distribution profiles on Days 2 and 3, display a further accumulation of LMW size material, presumably due to the catabolism of the residual HES remaining in the bloodstream following the preceding injection. The HES recovered from the intravascular space 24 h after each injection, consisted of molecules of a LMW size, concomitant with a further narrowing and slight shifting to material of a larger molecular size distribution. Four hundred and eighty hours after the third and final injection (Figure 2), the HES recovered from the circulation, was composed of molecules possessing an intermediate and narrow size distribution, between LMW and HMW size material. This narrow peak of HES molecular size composition, may possibly reflect a *steady-state equilibrium* between catabolism on the one hand, and elimination on the other.

Discussion

In man, the disappearance of HES (M_w 450,000 daltons, 70% hydroxyethylation) from the circulation following a single injection, is compound

and can be resolved into three exponential components; fractions with slow (IT_{50} 67 h), intermediate (IT_{50} 6.1 h) and rapid (IT_{50} 0.86 h) clearance rates (Metcalf *et al.*, 1970). In subjects given consecutive daily infusions of HES, the removal of this material from the intravascular space on all days, was best described mathematically by a series of exponential equations, rather than by linear regression analysis (Mishler, 1978). However, linear regression analysis adequately ($r^2=0.85$) described the clearance of HES from the bloodstream, during the post-injection period following the final infusion. The complex nature of this intravascular removal is certainly due in part to the heterogeneous distribution of varying molecular weight populations (10,000 to 1,000,000 daltons) contained in the injected solution. More recent studies have shown that hydroxyethylation of the 2-carbon on the anhydroglucose units of HES may be more resistant to α -amylase degradation than substitution on the 6-carbon (Yoshida, Yamashita, Matsua & Kishikawa, 1973). The hydroxyethylation, however, does not completely inhibit α -amylase mediated hydrolysis, because sugar can be generated on incubation of HES with whole blood (Thompson, Britton & Walton, 1962) or saliva (Farrow *et al.*, 1970). The two major properties of HES thus shown to significantly influence intravascular persistence, are weight average molecular weight (M_w) and the degree of substitution (hydroxyethylation) (Bogan *et al.*, 1969; Thompson *et al.*, 1962).

Gel filtration had previously shown the extraordinarily wide molecular size distribution of the HES injected solution (Farrow *et al.*, 1970), and these observations have been confirmed in the present study

(Figures 1 and 2). The distribution of molecular size in HES recovered from the circulation 10 min after each injection was much narrower than the distribution in the HES originally injected (Figure 1). Smaller molecules had been removed, presumably by filtration at the glomerulus. This observation is taken from two considerations; firstly, the number average molecular weight (M_N) of the injected HES solution is approximately 71,000 daltons, and thus one would expect a large fraction of this material to fall below the glomerular threshold and be excreted in the urine. Gel filtration has further confirmed that the molecular composition of excreted HES, consisted of a narrow distribution of small molecular size material (Bogan *et al.*, 1969). Secondly, the urinary excretion rate (g h^{-1}) during the first post-injection h is high following single (Metcalf *et al.*, 1970) and repeated (Mishler *et al.*, 1977b) HES infusions, while the rate of excretion after this period falls markedly. The distribution profiles 10 min post-injection on Days 2 and 3, display a further accumulation of LMW size material, presumably due to the hydrolysis of the residual HES remaining in the intravascular space following the preceding infusions. It has been previously demonstrated in man that approximately 57–65% of the total quantity of HES measured 10 min post-injection following consecutive daily infusions, remains in the circulation after 24 h (Mishler *et al.*, 1977a). The catabolism of this residual HES fraction, would thus explain the increase in LMW size material in the distribution profiles on Days 2 and 3. The HES contained in the sampled plasma 24 h post-injection (Figure 1), consisted of molecules possessing a LMW distribution, concomitant with a slight shift to molecules of a larger molecular size. These results would be consistent with the catabolism or removal of the HMW fraction, as indicated by the shifting of the molecular distribution to material of a LMW size. In addition to α -amylase mediated breakdown, the HMW fraction may be removed by cells contained in the reticuloendothelial system. This stems from two considerations; firstly, 24 h

after the infusion of HES (M_w 450,000 daltons, 70% hydroxyethylation), 20% of the total quantity infused is unaccounted for, either in the intravascular space or in the material excreted (Metcalf *et al.*, 1970). Secondly, labelling studies conducted in animals have shown that numerous organs and tissues, retain HES for various time intervals after infusion (Thompson, Fukushima, Rutherford & Walton, 1970). These observations tend to indicate that the gradual elimination of the HMW fraction contained in the injected HES solution, is brought about by a combination of α -amylase mediated hydrolysis and removal by certain viscera. Thompson & Walton (1963) have claimed, however, that tissue storage of HES in the major viscera of dogs was distinctly less than that of dextran.

The molecular size distribution 480 h following the third and final infusion (Figure 2), was distinct and narrow, indicating an HES molecular species between LMW and HMW size material. At this point in time, the intermediate molecular size profile may possibly represent a condition in which elimination and catabolism have reached a *steady-state equilibrium*. This would tend to be confirmed by the linear disappearance of HES from the intravascular space during the interval 24 to 480 h following the third and final infusion (Mishler, 1978). The distribution of hydroxyethyl groups among molecules is probably not uniform and may be determined by statistical considerations. It may thus be that those molecules remaining in the circulation during this post-injection period are likely to be those with the highest degree of substitution concomitant with the greatest resistance to hydrolysis by α -amylase.

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