Pharmacokinetics of Hydroxyethyl Starch in Normal Subjects

AVRAHAM YACOBI, Ph.D., ROGER G. STOLL, Ph.D., CHECK Y. SUM, Ph.D., CHII-MING LAI, Ph.D., SURINDER D. GUPTA, M.S., and JAMES D. HULSE, Ph.D. McGaw Park, Ill.

Abstract: To determine the elimination of high-molecular-weight hydroxyethyl starch (HES, $M_w$ 450,000) in normal subjects, ten volunteers were given 500 ml 6% HES solution by intravenous infusion, and serial blood and urine samples were collected for nonglucose total carbohydrate determination. On the average, 46 and 64 per cent of the dose was excreted in the urine within two and eight days, respectively. The plasma concentration declined rapidly during the first week after infusion. The average terminal half-life was 17 days during the first 42 days, which accounted for elimination of about 90 per cent of the dose. The remainder was eliminated with a terminal half-life of 48 days determined between days 42 and 83 of the study. As expected, the infusion of HES resulted in plasma volume expansion over a 48-hour period during which time levels of nonglucose carbohydrates were above 3.5 mg/ml. HES is metabolized by $\alpha$-amylase in the body. During the first 48 hours after infusion of HES, plasma $\alpha$-amylase activity was significantly increased over control. Concomitantly, $\alpha$-amylase activity in urine was also elevated but not significantly so.

Hydroxyethyl starch (HES, Hespan*) is a synthetic colloid plasma volume expander. In addition to its use as a volume expander, HES has been approved for adjunctive use in leukapheresis to increase the yield of granulocytes by centrifugal means. HES is a heterogeneous mixture of polysaccharides having a spectrum of molecular weights. The metabolism of such a mixture is a complex and dynamic process in which new but smaller HES molecules are continuously being formed. These smaller fragments are subject to distribution into organs or excretion in urine or bile just like the original molecules. Conflicting data exist in the literature describing elimination of HES in man. Therefore, elimination of HES was determined in a rigorously controlled study on 10 normal subjects in which urine and plasma samples were collected for up to 8 and 83 days, respectively.

In man* and laboratory animals,2,3 elimination of HES molecules from the body was shown to be dependent on the average molecular weight ($M_w$) and molar substitution (MS), i.e., the mole fraction of hydroxyethyl groups per anhydroglucose unit. Because of its heterogeneous nature, the kinetics of elimination for HES are complex. While smaller molecules ($M_w \leq 50,000$) are readily excreted in the urine, elimination of larger molecules depends on: (a) distribution into body tissues; (b) back-diffusion from tissues into intravascular spaces; and (c) metabolism by $\alpha$-amylases in blood, tissues, and the reticuloendothelial system, followed by urinary and biliary excretion of the products.

From The Research and Development Department, American Critical Care, 1500 Waukegan Road, McGaw Park, Ill. 60085.

* American Critical Care, McGaw Park, Ill.
Urinary excretion of HES in man has been studied in a few investigations. Metcalf et al. reported that after administration of HES to patients, 51 per cent of the dose was eliminated in urine within three days. In another study, four normal subjects receiving three consecutive daily doses of HES eliminated 41 to 46 per cent of the total dose in the urine after seven days. In a controlled mass balance study in rats, 60 and 74 per cent of an intravenous dose of \(^{14}\)C-HES was eliminated after 8 and 28 days, respectively. During the same respective periods, 8 and 11 per cent of the dose was eliminated in feces.

Metcalf et al. calculated a biologic half-life of 67 hours in man. In another study, 9 per cent of an administered dose of HES was detected in plasma after two weeks and greater than 1 per cent was detected after 17 weeks. An extrapolation of the data from these volunteers showed a half-life of about 30 days. In a more recent investigation, a half-life of 48 days was reported. In view of conflicting data in the literature, it was considered of interest to study (a) the elimination of HES into urine of normal subjects under controlled conditions; (b) the effect of time on elimination half-life of HES; and (c) the relationship between volume expansion and HES plasma concentrations.

Methods

Protocol

Ten healthy, nonobese, male subjects 22 to 33 years old and weighing 63.5 to 90.0 kg (mean 76.4 kg) participated in this study. All subjects were paid and signed a written consent form.

Each subject received 500 ml Hespan (6% hydroxyethyl starch, \(M_r 450,000 \) and \( M_S 0.7 \), in 0.9% sodium chloride solution, Lot no. B8HO87A, American Critical Care, McGaw Park, Ill.) by intravenous infusion over a 1-hour period. The subjects were hospitalized for eight days to ensure complete urinary collection during this period. They were provided a standard diet. Coffee, tea, or other caffeine-containing beverages and alcohol were not allowed during the hospitalization period as prudent measures.

Serial blood samples were drawn from all subjects for up to 28 days after administration of HES. Subjects 1, 5, 6, and 8 consented to participating in the study for up to 83 days. Daily urine samples were collected during the eight days of the hospitalization. Plasma and aliquots of urine samples were frozen immediately until assayed. An additional six subjects were entered into the study and hospitalized for 48 hours. These subjects received no Hespan, but serial blood samples were drawn for determination of baseline plasma carbohydrate concentrations and a-amylase activity. The average levels of non-HES, nonglucose plasma carbohydrates and a-amylase activity found in these subjects served as control values for this study. The values obtained from these six control subjects were in good agreement with the single control value obtained, prior to infusion of HES, from each of the 10 subjects.

Analytical Procedures

Nonglucose total carbohydrate levels in plasma and urine were determined by treating all samples with glucose oxidase to destroy endogeneous glucose prior to the anthrone reaction. Glucose levels in plasma and urine were determined via the reaction of glucose with glucose oxidase and using peroxidase to quantitate the hydrogen peroxide formed. Plasma albumin levels were determined by the binding reaction of brom cresol green with albumin, and total protein levels were determined by the Folin-Lowry method. The a-amylase activity in plasma and urine was determined by quantitating the amount of reducing sugars formed after incubating soluble starch with plasma or urine samples. The plasma volume expansion after administration of HES at a given time (PV<sub>i</sub>)
was determined by the method described by Metcalf et al:\(^1\):
\[ PV_t = (PV_0 - S) \text{ albumin}, \]
where \( PV_0 \) is the initial plasma volume before HES administration (calculated by the method of Dagher et al.\(^1,^2\)) and \( S \) is a correction factor for the volume of plasma removed during blood sampling.

Results

Figure 1 shows the average cumulative excretion of HES in urine. An average of 46 and 64 per cent of the dose was excreted after two and eight days, respectively. The average urinary glucose levels in these subjects were below 0.2 mg/ml. The average plasma concentrations-versus-time profile of HES is shown in Fig. 2. Data from another human study,\(^5\) in which different study conditions existed and a different lot of HES was administered, are also shown. Even though HES concentrations were determined using the same method, data from the other study\(^8\) appeared to be consistently higher than those observed in the present study. In this study, the average plasma concentration declined to less than 47 and 23 per cent of the peak value (observed at 1 hour) after two and eight days, respectively. The average baseline plasma concentration was less than 3 per cent of the
peak value after 63 days. There were no adverse reactions related to HES in this study.

Because of the heterogeneity of HES, its elimination cannot be described by a conventional model. The half-life of HES was estimated over linear segments of the plasma concentration-versus-time profile at the postinfusion-postdistribution phase. Table I summarizes the estimated biologic half-life of HES during different periods of elimination. The biologic half-life increased with time. When data from 14 to 42 days were analyzed, the half-life was 17 days. When plasma concentrations beyond 42 days (in four subjects) were analyzed, the half-life increased considerably and reached a plateau at 48 days.

Figure 3 depicts the relationship between plasma volume expansion and HES concentrations in plasma. The plasma volume increased by about 9 per cent after administration of 500 ml 6% HES solution and returned to its baseline level within 48 hours (at which time the plasma HES concentration was 3.5 mg/ml). There was a significant correlation between the ratio of plasma volume at time t divided by the control value ($PV_t/PV_0$) and the logarithm of HES plasma concentration at that time ($r = 0.940$, $P < 0.001$).

Figure 4 shows that plasma $\alpha$-amylase activity increased approximately twofold 24 hours after administration of HES. The plasma $\alpha$-amylase activity was statistically higher ($P < 0.05$) than the baseline activity during the period of 1.1 to 48 hours after infusion. The plasma profile of increased $\alpha$-amylase activity is consistent with previously reported data. There was also an increase (but not statistically significant) in urinary excretion of $\alpha$-amylase during this time period. This elevation of $\alpha$-amylase in the plasma and a decreased protein concentration due to volume expansion were the only changes noted in blood chemistries.

**Discussion**

Hydroxyethyl starch (HES) is a heterogeneous mixture of polymers differing basically in chain length and molecular weights. The metabolism of such a mixture is a complex and dynamic process in which new but smaller HES molecules are continuously being formed. These smaller fragments are subject to distribution into organs or excretion in urine or bile just like

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**TABLE I**

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Half-life (days)</th>
<th>Half-life values determined from and beyond indicated time (days)</th>
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<tr>
<td></td>
<td>7-28</td>
<td>14-42</td>
</tr>
<tr>
<td>1</td>
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<td>19.3</td>
</tr>
<tr>
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<td>18.5</td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
<td>Mean</td>
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</tr>
<tr>
<td>S.D.</td>
<td>±2.16</td>
<td>±4.46</td>
</tr>
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</table>
HES was excreted in feces by man (unpublished data), which suggests a species difference in the biliary excretion of HES.

In order to accurately determine the plasma concentration of HES, a knowledge of the baseline level of non-HES carbohydrate is needed. To accomplish this, a group of six subjects who did not receive any HES, but received the same diet as the other group, were included in the study. The analysis of serial plasma samples from these subjects showed baseline carbohydrate levels ranging from 0.022 to 0.121 mg/ml, with a mean and standard deviation of 0.047 ± 0.019 mg/ml, over a 48-hour period. These values indicate that great caution should be used when measuring plasma HES levels below about 0.25 mg/ml, particularly if meaningful pharmacokinetic analyses are being considered. In fact, 49 to 83 days after administration of

the original molecules. Conflicting data exist in the literature describing elimination of HES in man. Therefore, elimination of HES was determined in a rigorously controlled study on 10 normal subjects in which urine and plasma samples were collected for up to 8 and 83 days, respectively.

There is a striking similarity in urinary excretion of HES in normal subjects who participated in this study and in rats which received a 0.9 Gm/kg dose of 14C-HES as part of a mass balance study. In both studies, HES was prepared by essentially the same procedure and met the same specifications. In eight days, rats eliminated 63 per cent of the dose in urine which is comparable to the 64 per cent obtained in this study. During the same period, 9 per cent of the dose was eliminated in feces of the same rats. On the average, less than 1 per cent

**Fig. 3.** Relationship between plasma volume expansion and plasma HES concentration in 10 normal subjects who received 500 ml 6% HES intravenously during the first 48 hours after termination of infusion.

**Fig. 4.** Average plasma and urinary α-amylase activity in nine normal subjects who received 500 ml 6% HES intravenously. There was a significant difference (<0.05) from baseline activity for plasma samples 1.1 to 48 hours after initiation of the infusion. One unit of α-amylase activity is expressed as that amount of enzyme that will form 1 μmole maltose in 20 minutes at 37°C. Only nine subjects were used, as subject 6 had a pre-dose plasma α-amylase activity 2.7 times higher than the mean of the other nine subjects.
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HES, plasma levels of carbohydrates found in this study were not significantly different from baseline levels. However, this finding is based on a very small number of subjects.

Because the biologic half-life of HES changes as a function of time, it was decided to analyze linear segments of the plasma concentration-versus-time profiles. Although this approach does not represent a true terminal half-life, it does provide some information regarding elimination of HES. Using this approach, one can estimate that HES has a terminal half-life of 17 days during the first 42 days after administration. Using that half-life, and on the basis of the remaining quantity of HES in the body (36 per cent after eight days), it is reasonable to assume that as much as 90 per cent of the dose is eliminated within the same period.

In rats, nearly 90 per cent of the dose is eliminated within 28 days with a terminal half-life of 18 days. In man, 64 per cent of the dose is eliminated in urine within eight days, but the plasma concentrations declined from 1.6 mg/ml (21.2 per cent of the peak level) on day 8 to 0.34 mg/ml (4.5 per cent of the peak level) on day 42, an almost fivefold decrease. A small quantity of HES, approximately 10 per cent, which is well distributed in the tissues, will be eliminated with a half-life of 48 days, which is in excellent agreement with the value obtained for the terminal half-life of HES in normal subjects in a different study.

The increase in \( \alpha \)-amylase activity in plasma is interesting since it also corresponds with an increase in urinary excretion of the enzyme. Because there is a large variation in the amount of \( \alpha \)-amylase excreted in the urine, no statistically significant difference from the baseline was detected. The increase in plasma \( \alpha \)-amylase activity has been postulated to be due to an interaction between \( \alpha \)-amylase and HES which results in decreased urinary excretion of \( \alpha \)-amylase. The results of this study suggest the possibility of another mechanism—simply an increased release of \( \alpha \)-amylase from different tissues as a result of higher concentrations of HES.

In this study, a relatively small dose of HES was administered which resulted in a 9 per cent increase in plasma volume in normal subjects. There is a striking correlation \( (r = 0.940, P < 0.001) \) between volume expansion and plasma concentration of HES above 3.5 mg/ml in these subjects. Since HES is usually given after hemorrhage and loss of large quantities of fluid and proteins from the body, a similar relationship between volume expansion and plasma HES levels in patients may also exist, except that changes in plasma volumes may be greater.

This study has shown that a large fraction of an administered dose of HES is eliminated in the initial period following infusion. During this same period of time, there was a plasma volume expansion and an increase in plasma \( \alpha \)-amylase activity. As \( \alpha \)-amylase metabolizes the HES, plasma levels of HES decrease, the \( \alpha \)-amylase activity declines, and the plasma volume expansion diminishes. The chemical nature of the fraction that remains in the body over an extended period of time remains to be elucidated.

A long half-life usually is the reason for accumulation of drugs and/or their metabolites in the body. Good examples of such drugs are the phenothiazines and their metabolites. Since HES is not generally used for chronic or frequent administration, little accumulation of the drug is expected. In addition, HES has been used in numerous applications with low incidence of side effects.

References


Address correspondence to: Avraham Yacobi, Ph.D., Section Head, Clinical Pharmacology and Drug Metabolism, Pharmaceutical Development Department, American Critical Care, 1600 Waukegan Road, McGaw Park, Ill. 60085.