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Electrophoretic analysis of hydroxyethyl-starch induced macroamylasemia

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SUMMARY

Various species of hydroxyethyl-starch (HES) has been developed for use in the management of hypovolemia, and intravenous infusion of HES results in iatrogenic macroamylasemia. Clinically, recognition of macroamylasemia is important to distinguish them from other conditions that are attended by hyperamylasemia. Electrophoresis is a generally applicable test for detection of naturally occurring macroamylase, however, there is no information of about the detection of the iatrogenic macroamylase by electrophoretic analysis. We examined the serial amylase isoenzymes electrophoretic patterns of serum in 23 patients undergoing abdominal surgery. In them, 12 patients were dosed with 500-1,000 ml of 6% solution of the HES possessing a relative molecular mass of 30,000-40,000 combined with a molar hydroxyethyl group substitution of 0.50-0.55 upon operation. Electrophoretic analysis of the sera revealed that HES resulted in the characteristically anomalous electrophoretic pattern with cathodally migrating broaden amylase bands.

Key words : hydroxyethyl-starch (HES) induced macroamylase, iatrogenic macroamylase, amylase isoenzyme electrophoresis.

INTRODUCTION

Macroamylase is an enzymatically active macromolecular complex whose large size precludes its excretion in the urine and may result in hyperamylasemia. Macroamylase occurs in two forms. Naturally occurring macroamylase is an amylase-immunoglobulin complex and most of this macroamylase persistently exist in serum.¹⁾ Iatrogenic macroamylase is produced by interaction of amylase with hydroxyethyl starch (HES) following infusion of the latter compound into the blood stream.²⁾ This form of macroamylase is transient.

The various molecular weight species of HES have been utilized clinically as a plasma volume expander in the management of hypovolemia. The major portion of the injected HES in the blood stream is hydrolyzed to smaller polymer fragments, which are subsequently eliminated by the kidneys,³⁻⁵⁾ and a portion of the injected HES is taken up by the reticulo-endothelial system.³⁾

Electrophoresis is a generally applicable test for naturally occurring macroamylase that can be readily performed by most clinical laboratory.⁶⁾ Even though the HES induced macroamylase has been confirmed by gel permeation analysis of serum, there is no information about the detection

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of this macroamylase by electrophoretic analysis. The purpose of this study is to know the pattern of the HES induced macroamylase when analyzed by an electrophoretic isoenzyme procedure.

MATERIALS AND METHODS

Materials

A total of 23 patients undergoing abdominal major surgery was prospectively studied. Blood specimens were obtained from all patients before operation, and at intervals during the first 24 h after initiation of the operation. In them, 12 patients received by intravenous infusion, over a 30 to 90 min period, 500 or 1,000 ml of a 6% (w/v) solution of HES (HESPANDER® , Kyorin Pharmaceutical Co., Ltd., Tokyo, JPN : relative molecular mass (Mr) of 30,000-40,000, molar hydroxyethyl group substitution (MS) of 0.50-0.55) after hemorrhage upon the operation. The other 11 patients did not receive the HES before, during and after the operation. In the total of the patients the values for preoperative serum urea and creatinine were normal.

Serum amylase activity

Total serum amylase activity was determined on an automated-analyzer (Clinalyzer JCA-RX 30, Japan Electron Optical Laboratory, Tokyo, JPN) by use of a coupled enzymatic reaction sequence with maltopentaose as a substrate (Dia Colour AMY Rate, Ono Pharm., Osaka, JPN). Normal reference values range between 40 and 160 U/l.

Electrophoresis

Amylase isoenzyme electrophoresis was separated on cellulose acetate plate (TITANIII Lipo, Helena Labs., Beaumont, TX, USA) using 0.34 M tris-glycine (pH 8.9-9.3) as anodal buffer and 0.05 M barbital-sodium-boric (pH 8.7-9.1) as cathodal buffer (Helena Labs.). From 0.3 to 1.2 μl of samples was applied to the plate, depending upon total serum amylase activity. The plate was electrophoresed in a room temperature for 50 min at 300 V. After electrophoresis, the plate was overlaid with blue starch suspension (Daiichi Pure Chemicals, Tokyo, JPN) and incubated at 37°C for 30 min.

Gel-exclusion high-performance liquid chromatography (HPLC)

HPLC analysis was carried out on a Pharmacia

automated fast-protein liquid chromatography system (Uppsala, SWED) with a Superose 12 HR column (30 cm×1.0 cm I. D.) equilibrated with 0.15 M sodium chloride, 0.05 M phosphate buffer (pH 7.2). Protein was monitored at 280 nm, and amylase activity was monitored with use of an amylase kit (Iatron Labs., Tokyo, JPN). For the determination of the Mr of amylase, the following standard proteins (Pharmacia) were used : ferritin (Mr 440,000), catalase (Mr 232,000), aldolase (Mr 158,000), bovine serum albumin (Mr 67,000), ovalbumin (Mr 43,000), chymotrypsinogen A (Mr 25,000) and ribonuclease A (Mr 13,700).

Fig. 1. Changes in the amylase isoenzymes electrophoretic pattern in serum of a surgical patient, infused with 500 ml of a 6% solution of HES (HESPANDER®) upon operation, before, during and after the operation.

Numbers refer to collection time (in hours) after initiation of the operation.* and **, initiation and termination of the infusion; ***, termination of the operation; P, pancreatic amylase; S, salivary type amylase; O, serum application point.

Determination of amylase-immunoglobulin complexes

Presence of amylase-immunoglobulin complexes was examined by immunoprecipitin reactions in free liquid media method⁷⁾ using anti- γ chains, anti- α chains, anti- μ chains (Dakopatts, Gloustrup, DEN), anti- κ chains and anti- λ chains antiserum (Silenus Labs. Victoria, AUS).

RESULTS

1. Changes in serum amylase isoenzyme electrophoresis induced *in vivo* by infusion of HES

Serial electrophoretic patterns of serum from a patient (case 6 in Fig. 2) received 1,000 ml of the

HES solution within 60 min are shown Fig. 1. The sera obtained prior to operation and before the infusion upon the operation demonstrated typical amylase bands, however, the sera obtained during the course of the infusion period and the first 4.7 h following termination of the infusion revealed anomalous bands: cathodally migrating broaden pancreas type amylase main band (P1) with smearing of amylase activity between P1 and salivary type amylase main band (S1). The broadest band appeared 1.5 h post-infusion (i. e., after termination of the infusion). By 17 h post-infusion, no more the cathodal migration was observed in the obtained serum. No amylase-immunoglobulin complex was detected in the

Fig. 2. Results from serial analysis of serum amylase isoenzymes electrophoresis in 12 surgical patients after initiation of infusion of the HES solution.

↓, termination of the infusion; ●, anomalous broaden bands; ○, usual bands. Numbers represent values for serum amylase activity (U/l).

Fig. 3. Effects of HES on amylase isoenzymes electrophoretic pattern.

A: two-fold diluted serum from a patient having pancreatitis was mixed in equal volumes with a saline (1), the HES solution (2), the solution concentrated 2-fold (3), 4-fold (4), 6-fold (5) and 8 fold (6), respectively. B: Mixtures (1: 1) of two-fold diluted serum from a patient having salivary type hyperamylasemia. A number similar to Fig. 3 A.

sera obtained before the infusion, 1.5 and 17 h post-infusion. The anomalous changes in the amylase isoenzyme electrophoresis of serum were observed in all of 12 patients infused with the HES, as shown in Fig. 2. The anomaly was detectable during the infusion and for up to approximately 5 h post-infusion. The peak of the anomaly appeared within 2 h after initiation of the infusion. Sera recovered beyond 6.5 h post-infusion revealed the usual amylase bands. By contrast, of the patients were not given the HES, none demonstrated the anomaly.

There was a steady increase in serum amylase activity during the first 6 h post-infusion in 8 of the 12 patients. The isoamylase mainly responsible for the increase was not estimated, because it was impossible to distinguish between cathodally migrating broaden isoamylase bands on the electrophoresis. In the ranged from 15 to 20 h after initiation of the infusion, 10 (83%) of the 12 patients revealed hyperamylasemia. Of the 10 patients, the amylase elevation was mainly of the pancreas type in 4 (case 4, 7, 9 and 10), mainly of the salivary type in 3 (case 3, 5 and 11) and of both the type in the other 3 (case 1, 6 and 8). By contrast, 2 (18%) of the 11 patients without the

infusion showed a steady increase in serum amylase and finally hyperamylasemia. The amylase elevation was mainly in the pancreas type in both the patients.

2. Changes in serum amylase isoenzyme electrophoresis induced *in vitro* by incubation of HES with the serum

For this experiments, we used serum from a patient with pancreatitis (amylase activity, 558 U/l) and serum from a patient with salivary type-hyperamylasemia (amylase activity, 600 U/l). Each of the sera was diluted to two-fold of its volume and mixed in the ratio of 1: 1 with the HES solution concentrated to different extend of its volume. The *in vitro* mixtures were incubated at room temperature and electrophoresed after 5 min. The activity of amylase was essentially unchanged in the mixtures. The electrophoretic patterns of the mixtures resembled closed those of the sera from patients given the HES infusion and there was dose-dependent broadening of both pancreas type bands and salivary type bands (Fig. 3).

3. HPLC analysis of macroamylases induced, *in vivo* and *in vitro*, by HES

In this experiment, we used sera from a patient

Fig.4. Elution patterns of amylase activity during HPLC of samples with or without macroamylase induced *in vivo* and *in vitro* by HES.

0.2 ml of each sample was injected and eluted at 0.4 ml/min with 0.15 M sodium chloride, 0.05 M phosphate buffer (pH 7.2). The protein absorbance (-) and the amylase activity (Δ , $\blacktriangle$, $\bullet$, $\circ$) were monitored at 280 nm and 600 nm, respectively. A: Serum samples from a patient (case 3) infused with the HES. Δ and $\blacktriangle$, 2 h and 6.5 h after termination of the infusion of the HES, respectively. B: *In vitro* sample of an equal volume mixture of normal serum and the four-fold concentrated HES ($\circ$); $\bullet$, the normal serum without addition of the HES. The elution position of normal serum amylase (peak 4) was indicated by the arrows. 1, 2 and 3 were macroamylase peaks.

(case 3) drawn at 2 and 6.5 h post-infusion and an mixture of an equal volume of the normal serum and four-fold concentrated the HES solution. These samples were frozen at -20°C and incubated at room temperature prior to HPLC analysis (Fig. 4 A and 4 B). In the patient's serum drawn at 2 h post-infusion, showing the broaden bands, a large peak of macroamylase (peak 2) and normal amylase peak (peak 4) were noted: the corresponding retention time were 32.0 min and 41.0 min, and apparent Mr were 98,000 and 12,500, respectively. In the same patient's serum drawn at 6.5 h post-infusion, showing the usual bands, two macroamylase peaks (peak 2 and 3) were noted and their corresponding retention time were 32.0 min and 37.5 min, and the apparent Mr were 98,000 and 25,000, respectively. In the mixture, a single large peak (peak 1) was noted and the corresponding retention time was 25.5 min and the apparent Mr was 340,000.

DISCUSSION

The clinical importance of macroamylase is in resolving hyperamylasemia of unknown origin. Although the increased molecular mass of macroamylase is most readily determined by conventional gel permeation analysis,⁸⁾ the gel permeation, once used widely in clinical laboratories for the detection of naturally occurring macroamylase, has largely been replaced by amylase isoenzyme electrophoresis. The reason is that the characteristics of naturally occurring macroamylase upon the electrophoresis have been defined.^{9,10)} Also, in the use of gel permeation, the long fractionation time is needed. The major finding of this study makes the electrophoresis an appropriate initial test for detection of HES-induced macroamylase. Electrophoresis of HES-induced macroamylase produced cathodally broaden amylase bands. The cathodal migration with HES-induced macroamylase is comparable to that seen with IgG binding naturally occurring macroamylase.¹⁰⁾ So serial determinations are necessary if one is to distinguish HES-induced macroamylasemia from IgG binding macroamylasemia. HES-induced macroamylase is transient.

In the use of gel chromatography to estimate a

Mr of amylase, amylase-gel interaction occurs, resulting in a retardation of passage of amylase through the gel column⁸⁾ and hence a lower apparent Mr. Although the interaction render the HPLC method unreliable for accurate Mr estimation, this aids in the separation of normal-sized amylase from macroamylase.¹¹⁾ The apparent Mr value for macroamylase induced *in vitro* by the present HES estimated by the HPLC method was 340,000. The macroamylase originally appearing in circulation is broken down to of an apparent Mr 95,000, and even to of an apparent Mr 25,000 with time. This break down is still continuing at 6.5 h post-infusion. The duration of the persistence of HES-induced macroamylase in the circulation is related to the overall rate of HES catabolism, and the hydrolysis of HES is influenced by both the Mr and the MS of HES. A reduction in the Mr of HES has no apparent influence on the appearance of macroamylase complex formation.¹²⁾ The Ms influences the duration of complex formation, by its effects on modifying the catabolism of HES.¹³⁾ The macro-molecular mass of macroamylase induced by species of HES of MS 0.43,¹³⁾ of MS 0.60¹⁴⁾ and of MS 0.70¹⁵⁾ have been able to detect up to 6, 24 and 72 h post-infusion, respectively. The present surgical patients were dosed with the species of HES of Ms 0.50-0.55. The electrophoresis indicated the presence of macroamylase in the serum drawn up to 5 h post-infusion, but not in the serum drawn at 6.5 h post-infusion possessing macroamylase. For detection of HES-induced macroamylase, the sensitivity of electrophoresis is lower than gel permeation, however the electrophoresis is universary applicable test.

The infusion of HES results in hyperamylasemia.^{2,13,16)} For the pathogenesis is essential that the formation of macroamylase impairs the elimination of amylase without a disturbance of the eliminating organs.²⁾ In the present study, 10 (83%) of the 12 patients revealed hyperamylasemia in the period ranged from 15 to 20 h after initiation of the infusion. By contrast, of the 11 patients without the infusion, 2 (18%) revealed hyperamylasemia. Whether the hyperamylasemia in every patient given the HES

results from the formation of HES-induced macroamylase is not estimated, because post-operative hyperamylasemia occurs often and the peak of amylase levels usually appears about 24 h after operation.¹⁷⁾ In addition, we did not investigate the presence of macroamylase in the sera drawn in the period ranged from 15 to 20 h after initiation of the infusion. Further studies are necessary to elucidate this point.

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要 旨

Hydroxyethyl-starch (HES) 製剤は血漿増量剤として主として大量出血時に投与される。投与された HES は血中アミラーゼと複合体を形成し、医原性マクロアミラーゼを生じる。血中の自然発生するマクロアミラーゼ、つまりアミラーゼ-免疫グロブリン複合体と同様にこのマクロアミラーゼもゲルろ過法で確認することができるが、ゲルろ過法は簡便さに欠ける。自然発生マクロアミラーゼの存在は特徴ある血清アミラーゼアイソザイム電気泳動パターンから簡単に知られるが、この医原性マクロアミラーゼ血症の電気泳動分析に関する情報はない。今回、23 人の手術患者（内 12 人に HES が投与された）の手術前、中、後の血清アミラーゼアイソザイム電気泳動分析結果からこの医原性マクロアミラーゼも特徴ある血清アミラーゼアイソザイムパターンをきたすことを見いだした。