NOTE

A Screening Test to Detect HGH-Antibodies in Pituitary Dwarfs Treated with HGH Preparation by the Radioimmunoassay with Double Antibody Technique

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Synopsis

Sixty-four blood samples, obtained from 25 pituitary dwarfs treated with KABI-HGH preparation, were checked for serum HGH concentration by a double antibody radioimmunoassay and for antibodies against HGH by the method of propylene-glycol separation. Antibodies to HGH were detected in the samples whose HGH concentration was falsely estimated to be more than 5 ng/ml, but not in those whose HGH concentration was 5 ng/ml or less. The measurement of HGH concentration by a double antibody technique in the serum obtained from pituitary dwarfs 3-4 days after the last injection can be used to screen the presence of antibodies to HGH preparation used.

Since HGH treatment of pituitary dwarfs was introduced, antibody formation to HGH has been widely discussed (Parker *et al.*, 1964; Roth *et al.*, 1964; Quabbe *et al.*, 1967; Tanner and Whitehouse, 1967; Illing *et al.*, 1970; Underwood *et al.*, 1974). The procedure to detect antibodies in patients treated with HGH was originally based on chromatoelectrophoresis (Roth *et al.*, 1964). It is, however, troublesome to check for antibodies in all samples routinely by this technique. This paper deals with a method to presume the presence of antibodies by means of measuring the serum HGH level of treated patients using a commercially available kit of HGH radioimmunoassay based on a double antibody method, which is the most popular technique in Japan.

Patients and Methods

Twenty-five euthyroidal pituitary dwarfs were treated with KABI-HGH for a period of 6 months to 3 years and blood samples were monthly obtained 3-4 days after the last injection of HGH. The HGH preparation was intramuscularly administrated in doses ranging 0.25 to 0.5 IU/kg of body weight/week. The serum was stored at -20°C until analyzed for antibodies to HGH and HGH concentration. The serum HGH level was assayed by a double antibody radioimmunoassay using Dainabot kit. HGH antibodies in the serum were checked by a single antibody technique using polyethylene-glycol (PEG). Serum samples (0.1 ml) were incubated with 0.1 ml of ¹²⁵I-HGH (approximately 10,000 cpm) in a total incubation volume of 0.7 ml, added with 0.5 ml of borate buffer (0.5 M, pH 8.6) containing 2.5% BSA. The tubes were kept at 37°C for 24 hr. The samples were diluted routinely to 1:10, to 1:100 and 1:1000 when it was necessary. Separation of the bound from free ¹²⁵I-HGH was accomplished by PEG 20000. Forty gram of PEG was dissolved in 100 ml of borate buffer (0.5 M, pH 8.6) and mixed for one hr with magnetic stirrer. Five tenth ml of PEG solution

was added to each incubation tube and the mixture was centrifuged at 3,000 rpm for 20 min. The supernatant was removed with an aspirator and radioactivity of the precipitate was counted.

When ^{125}I -HGH, antibody-free serum and excess antibody of our radioimmunoassay were incubated, 'bound ^{125}I -HGH' separated from 'free' by the PEG method was 70%, while ^{125}I -HGH non-specifically bound to PEG was 20%. Therefore, the titration of HGH antibodies were defined as the highest serum dilution binding 45% of the ^{125}I -HGH. When 'bound' was more than 20% but less than 45%, the presence of antibodies in the serum is strongly suspected, but the antibody concentrations in less than 1:1 titer were decided to be negative in our studies.

Results

As a preliminary experiment, immunological cross-reactivity was compared between Wilhelmi HGH of our radioimmunoassay and KABI-HGH used for treatment. When ^{125}I -HGH (Wilhelmi) was used as tracer, the inhibition of binding to HGH antibodies by KABI-HGH was 84% of that of Wilhelmi HGH. Two mg of HGH was given to 3 pituitary dwarfs 2 times at intervals of 48 hr. Serum HGH levels increased from less than 3.0 ng/ml to the maximum levels ranging from 15 to 32 ng/ml at 3 to 6 hr after the injection and decreased to the basal level at 48 hr in all cases studied.

Basal HGH levels of 25 pituitary dwarfs studied were less than 3.6 ng/ml before the treatment and no antibodies to HGH were detected. In a serial dilution experiment as shown in Fig. 1, antibodies were detected at 3 months of the treatment. The titer of HGH antibodies in case 2 was 1:10 at 3 months and developed to 1:200 at 6 months of the treatment.

Sixty-four samples obtained from 25 pituitary dwarfs during the treatment were assayed for the level of serum HGH and simultaneously for the HGH antibodies. HGH levels were less than 5 ng/ml in 47 samples and more than 5 ng/ml in the other 17 samples. Antibodies to HGH could be

detected in the latter but not in the former. Fig. 2 shows the relation between the falsely estimated HGH levels and the titer of antibodies. Dilution curves of 5 samples with falsely high HGH values were not parallel to the standard curve of the radioimmunoassay.

Discussion

A marked cross-reaction between KABI-HGH and Wilhelmi HGH made it possible to measure the KABI-HGH levels in the blood of patients injected with KABI-HGH by our radioimmunoassay using Wilhelmi HGH as a standard. Since the increased HGH level after the injection of KABI-HGH decreased to the initial levels in 2 days, blood samples to detect HGH antibodies were taken 3-4 days after the last injection.

It seems that antibodies produced in these patients react with ^{125}I -HGH *in vitro*, but in the case of the double antibody technique this soluble complex does not react with the second antibody, anti-guinea pig gammaglobulin goat serum, and remains in the supernatant after centrifugation. Therefore, the bound % in a double antibody technique is decreased when serum samples contain antibodies to KABI-HGH, and then falsely high results are obtained. The same is with TSH when endogenous antibodies to TSH are present (Chaussain *et al.*, 1972). Since HGH antibodies produced in the patients during the treatment seem to be bound to endogenous HGH *in vivo* and to HGH preparation *in vitro* (Illig, 1972), the measurement of serum HGH by a double antibody radioimmunoassay can be used as a screening test for the presence of its antibody in pituitary dwarfs treated with HGH preparations. If blood samples are taken 3-4 days after the last injection of HGH and the HGH levels are more than 5 ng/ml, they must be checked

Fig. 1. Serial dilutional experiment to detect antibodies against HGH in 9 treated pituitary dwarfs. Blood samples were obtained at 3 months of treatment. Antibodies in a titer of 1:10 were detected in 2 of the 9 patients and the titer of Case 2 developed to 1:200 at 6 months of the treatment.

for antibodies against HGH. This is a useful test for screening HGH antibodies in a laboratory where the routine radioimmunoassay for HGH measurement is a double antibody technique.

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Fig. 2. Relation between titers of antibodies and falsely estimated HGH concentrations. Sixty-four samples obtained from 25 pituitary dwarfs on HGH preparation were checked for the presence of antibodies against HGH and simultaneously for the serum HGH levels by a double antibody technique. Antibodies to HGH were detected with the titers ranging from 1:1 to 1:200 in 17 samples whose HGH concentrations were falsely estimated to be more than 5 ng/ml, but not in 47 samples whose HGH concentrations were less than 5 ng/ml.

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