TREATMENT OF PITUITARY DWARFISM WITH METHIONYL HUMAN GROWTH HORMONE IN JAPAN

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Abstract

Sixty-two patients with pituitary dwarfism were treated with three different preparations of methionyl hGH (m-hGH) for 3 to 14 months. They were given 0.5 IU/kg/week intramuscularly. The growth rate during treatment with the three different preparations was the same for each and increased from 3.5 ± 0.9 to 8.2 ± 1.7 cm/year. A high incidence of hGH antibody formation was observed following the treatment, but the titer of antibody was decreased according to the purity of m-hGH preparations. At the end of 12 month treatment with a highly purified preparation (Somatonorm III), 76.2% of the patients had hGH antibody. However, the presence of antibodies did not affect the growth rate except in one patient. No clinical or laboratory side-effects were observed following the treatment with m-hGH. Thus, m-hGH was considered to be useful for the treatment of GH deficient children.

Since the success of hGH synthesis by recombinant DNA technology (Goeddel et al., 1979), several investigators have reported the properties and biological activities of methionyl-hGH (m-hGH) in man and animals (Olson et al., 1981, Hizuka et al., 1982, Rosenfeld et al., 1982a, 1982b, Frigeri et al., 1982, Hintz et al., 1982). We have previously reported biological effects of the first preparation of m-hGH (Somatonorm® I) in normal healthy volunteers (Takano et al., 1983a) and in patients with GH deficiency (Takano et al., 1983b). In this study, we investigated the growth response and antigenicity of further purified preparations of m-hGH, Somatonorm II and III, in the treatment of patients with GH deficiency. Furthermore, we have studied the growth response and serum hGH antibody after switching Somatonorm I and II to pituitary extracted hGH in patients treated with Somatonorm I and II. This study
was performed between October of 1982 and February of 1986.

Materials and Methods

Patients and study design
The patients with GH deficiency consisted of 44 boys and 18 girls aged 4-16 years. The diagnosis of GH deficiency was according to the recognized criteria of this disease. Features of pituitary dwarfs used in this study:
1) Peak plasma GH level was less than 5 ng/ml to insulin induced hypoglycemia.
2) Their birth weight was more than 2500 g.
3) Their bone age was less than 11 years in boys and less than 10 years in girls (pre-pubertal).
4) They had no prior history of treatment with hGH or anabolic agents.
5) In cases where they had other hormone deficiencies, they were under suitable replacement therapy.

The aetiology of the GH deficiency in 52 patients was considered to be idiopathic, since all had normal lateral skull X-rays and normal CT scans. Seven patients were operated on for craniopharyngioma and one patient was operated on for cerebellar tumor at least one year before this study. Two patients had received Liniac and $^{60}$Co irradiation a year and a half before and four years before for their pinealoma, respectively. Each patient received 0.5 IU/kg/w of three different preparations of m-hGH in two to four divided doses per week by intramuscular injection for 3-14 months. The patients treated with m-hGH were switched to pituitary extracted hGH (p-hGH) in the same dosages after experimental treatment with m-hGH. The patients treated with Somatonorm I were those reported earlier (Takano et al., 1983b).

During the treatment, vital signs and height and body weight were checked by the same physician. Blood count, urinalysis, routine chemistries, and measurement of antibody to hGH were performed once a month during the treatment with m-hGH. After switching from m-hGH to p-hGH, the patients visited the hospital every two or three months to have the above factors rechecked. Bone age was evaluated both before and every 6 months after the treatment.

Informed consent was obtained from each subject and/or the parents, and the experimental protocol was approved by the ethical committees of participating medical schools.

Growth hormone preparations
The synthetic methionyl human growth hormone (m-hGH) of three different levels of purity was obtained from KabiVitrum AB, Stockholm, Sweden. They were named Somatonorm I, II and III®. Each vial contained 4 IU hGH (by RIA), 40 mg glycine and 1 mg Na-phosphate. Amounts of E coli protein (ECP) in Somatonorm I (batch No. 82412), II (batch No. 86975, 88234) and III (batch No. 81000, 81577, 88922) were reported to be 220, 30-7 and 4-3 ng per vial, respectively. The treatment with Somatonorm I was started in October 1982 in 10 patients for 3 months and that with Somatonorm II was started in December, 1983 in 15 patients for 6 months. The treatment with the highly purified preparation of Somatsonorm III was first started in November, 1984 in 21 patients for 12 months and again in June, 1985 in 16 patients for 6 months.

Assay method
Antibody to hGH was estimated by a polyethylene glycol procedure using serum obtained from patients. Incubation was performed with 50 $\mu$l $^{125}$I-hGH, 50 $\mu$l serum sample, 100 $\mu$l 1% $\gamma$-globulin, and with or without 50 $\mu$l of unlabelled hGH (1 mg/ml) in a total volume of 500 $\mu$l veronal buffer. Separation of bound and free hormone was performed using 500 $\mu$l of 25% polyethylene glycol (Besquiquois & Aurbach, 1971). Serum was diluted in a tenfold series and the titer of the antibody was expressed as the final dilution of the plasma which gave the optimal binding above the control. Control binding was obtained utilizing normal serum. As the labelled and unlabelled hGH, hGH preparation (NIAMDD-hGH-I-1; AFP-4793B) was used. Routine chemistries and blood count were measured by standard automated techniques.

Bone age was estimated according to the standards of Greulich and Pyle (1959). Student's $t$-test and paired $t$-test were used for statistical analysis.

Results
The growth rates before and during m-
hGH treatment are shown in Table 1 and Fig. 1. During 3 months of Somatonorm I treatment in 10 patients, height increased between 1.5 and 2.7 cm, which calculated out to between 6.0 and 10.8 cm/year with a mean of $8.7 \pm 0.6$ (M±SEM) cm/year. This value was significantly greater than that of pretreatment ($3.7 \pm 0.2$ cm/year, $p<0.001$). During 6 months of Somatonorm II treatment in 15 patients, height increased between 2.9 and 4.7 cm which calculated out to between 5.8 and 9.4 cm/year with a mean of $8.1 \pm 0.3$ cm/year. This value was significantly greater than that of pretreat-

### Table 1. The height increase and calculated growth rate (M±SEM) due to 3 and 6 months of m-hGH treatment.

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>3M Treatment</th>
<th>6M Treatment</th>
<th>12M Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm/3M</td>
<td>cm/y</td>
<td>cm/6M</td>
</tr>
<tr>
<td>Somatonorm I</td>
<td>10</td>
<td>2.3±0.1</td>
<td>8.7±0.6</td>
</tr>
<tr>
<td>Somatonorm II</td>
<td>15</td>
<td>1.8±0.1</td>
<td>7.0±0.4</td>
</tr>
<tr>
<td>Somatonorm III</td>
<td>16</td>
<td>2.2±0.1</td>
<td>8.9±0.5</td>
</tr>
<tr>
<td># III</td>
<td>21</td>
<td>1.9±0.1</td>
<td>7.9±0.6</td>
</tr>
</tbody>
</table>

Fig. 1. Growth rate before (B) and during m-hGH treatment (T) (Somatonorm I, II and III). The bar indicates mean±SD.

Fig. 2. Relationship between growth rate on the one hand and chronological age and bone age on the other at the start of the treatment •; Somatonorm I, ○; Somatonorm II, ▲; Somatonorm III.
ment (3.4±0.8 cm/year, p<0.001). During 6 months of Somatonorm III treatment in 16 patients, height increased between 2.6 and 6.1 cm which calculated out to between 5.2 and 12.2 cm/year, and during 12 months of Somatonorm III treatment in the other 21 patients, height increased between 4.3 and 12.1 cm. The mean growth rate during Somatonorm III treatment (N=37) was 8.1±0.3 cm/year, which was significantly greater than that of pretreatment (3.4±0.2 cm/year, p<0.001). The mean increase in height obtained during Somatonorm I, II and III treatment did not differ significantly. There was no relationship between the growth rate on the one hand and chronological age and bone age the other, taken at the start of treatment (Fig. 2).

The appearance of hGH antibody during m-hGH treatment is shown in Fig. 3. All patients treated with Somatonorm I got antibodies after 2 months of treatment. The antigenicity decreased in Somatonorm II and III, in the order of the purity of m-hGH. The percentage of patients who had the antibody at the end of 3 month treatment of Somatonorm I, II, and III, were 100%, 66.7% and 51.4%, respectively. At the end of 12 months treatment with Somat}

![Graph showing HGH antibody appearance during m-hGH treatment](image)

**Fig. 3.** HGH antibody appearance during m-hGH treatment
- ●; Somatonorm I,
- ○; Somatonorm II,
- ▲; Somatonorm III.

![Graph showing percentage of patients with hGH antibody](image)

**Fig. 4.** Percentage of patients who had hGH antibody at the end of 3 months, 6 months and 12 months of m-hGH treatment.
- Antibody negative; □.
- The titer of hGH antibody of 10; 10, 10^{-1}; 10^{-2}; 10^{-3}; 10^{-4}; 10^{-5}.

Somatonorm III, 76.2% of the patients had hGH antibody. The titer of hGH ranged 10 to 10^{5} as shown in Fig. 4. The high titer of
hGH antibody, $10^4 - 10^5$, was observed in 60% of the patients at the end of 3 months treatment with Somatonorm I. However it was observed only in 2.7% at the end of 3 months treatment with Somatonorm III and it increased gradually to 14.3% at the end of 12 month treatment. After switching from m-hGH to p-hGH, the antibody disappeared gradually as shown in Fig. 5. The antibody disappeared within one year in 11 of 13 patients who got antibody during the treatment with Somatonorm II. The antibody produced by Somatonorm I did not disappear easily and 4 of 10 still had the antibody at 2 and a half years after changing the treatment, although the titer of hGH antibody decreased from $10^4 - 10^5$ to $10^2 - 10^3$ in these four patients.

The growth rate during m-hGH followed by p-hGH treatment classified according to the titer of hGH antibody observed at the end of Somatonorm I and II treatment is shown in Table 2. The growth rates in the patients who had hGH antibody with a titer of $10$, $10^2 - 10^3$ and $10^4 - 10^5$ were $8.6 \pm 0.8$, $8.2 \pm 0.3$, $7.5 \pm 0.6$ cm/year in the first year of treatment and $7.7 \pm 0.4$, $7.6 \pm 0.4$ and $7.4 \pm 0.5$ cm in the second year of treatment, respectively. The growth rate did not change significantly with the titer of hGH antibody. The growth curves in 7 patients who had a high titer of antibody, from $10^4$ to $10^5$, at the end of Somatonorm

![Fig. 5. HGH antibody disappearance after switching from m-hGH](image)

Table 2. Growth rate (cm/year) during m-hGH followed by p-hGH treatment according to the titer of hGH antibody observed at the end of m-hGH (Somatonorm I and II) treatment.

<table>
<thead>
<tr>
<th>Titer of hGH Ab</th>
<th>No. of Patients</th>
<th>1st year m-hGH → p-hGH</th>
<th>2nd year p-hGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10$</td>
<td>4</td>
<td>$8.6 \pm 0.3$</td>
<td>$7.7 \pm 0.4$</td>
</tr>
<tr>
<td>$10^2 - 10^3$</td>
<td>11</td>
<td>$8.2 \pm 0.3$</td>
<td>$7.6 \pm 0.4$</td>
</tr>
<tr>
<td>$10^4 - 10^5$</td>
<td>8</td>
<td>$7.5 \pm 0.6$</td>
<td>$7.4 \pm 0.5$</td>
</tr>
</tbody>
</table>

(M±SEM)
There was no significant change in blood count, urinalysis or routine chemistries during and after the treatment of m-hGH. There were neither pyrogenic effects nor other side effects. During 6 months and 12 months of Somatonorm III treatment, mean bone age changed from $5.8 \pm 0.6$ to $6.4 \pm 0.2$ and from $6.2 \pm 0.7$ to $7.5 \pm 0.5$ years, respectively.

**Discussion**

Sixty-two children with GH deficiency were treated with three different preparations of methionyl-hGH (m-hGH; Somatonorm I, II and III). The mean growth rates (Mean ± SD) of $8.8 \pm 1.7$, $8.1 \pm 1.0$ and $8.1 \pm 1.9$ cm/year due to Somatonorm I, II and III treatment, respectively, did not differ significantly and when calculated together (N=62), the growth rate was increased from $3.5 \pm 0.9$ to $8.2 \pm 1.7$ cm/year by m-hGH treatment. This growth rate was similar to that of pituitary extracted hGH (p-hGH) treated children in Japan ($7.7 \pm 1.2$ cm/year) (Shizume, 1984). No significant side effect was noted.

The only difference between m-hGH and p-hGH was the greater antigenicity due to m-hGH. The antibody decreased as the purity of m-hGH increased. However, with the highly purified preparation of m-hGH, Somatonorm III, 76.2% of the patients had antibodies at the end of 12 month treatment. This incidence was greater than that observed in patients treated with p-hGH. Shizume et al. (1974) reported that p-hGH (Crescormone®; KABI-hGH) was less antigenic. We detected antibodies in 32 out of 320 patients treated with different preparations of p-hGH for more than six months; the incidence was 10%. At KabiVitrum AB in Stockholm, they measured the binding capacity (by Scatchard plot analysis) of 80 serum samples of m-hGH treatment and we have analyzed these data as shown in
Fig. 7. Relationship between titer and binding capacity of hGH antibody (●; Somatonorm I, ○; Somatonorm II, ▲; Somatonorm III).

Fig. 7. The binding capacity was lower in the antibodies produced by Somatonorm II and III, even if the titer of antibody was the same as that produced by Somatonorm I. The lower binding capacity in antibody produced by Somatonorm II, in addition to the lower titer, might explain the faster disappearance rate of this antibody compared with that of Somatonorm I. From the brief analysis of disappearance of the antibody, we observed that hGH antibody with a titer of less than $10^2$ disappeared within 6 months, and that with a titer of $10^3$ disappeared within one year. In spite of the formation of hGH antibody, growth rate did not decrease in 48 of 49 GH deficient children. Only one patient treated with highly purified preparation, Somatonorm III, had growth attenuation. The titer of hGH antibody was $10^5$–$10^6$ and its binding capacity measured by KabiVitrum AB was 2.4 mg hGH/L.

As to the antigenicity of m-hGH, it is now felt that a minute contamination of E. coli protein may act as adjuvant to enhance immunogenicity of m-hGH. The possibility that the extra amino acid of methionine residue acts in the production of the antibody is denied at present. The clinical trial of methionine free biosynthetic hGH, which has been carried out in Japan since December 1985, will provide further information on the antigenicity of biosynthetic hGH preparation.

Acknowledgements

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References


