Treatment of 46 patients with Turner’s syndrome with recombinant human growth hormone (YM-17798) for three years: a multicentre study

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Turner’s syndrome is a chromosomal disorder involving many physical abnormalities; these include short stature and slow growth rate. Short stature has been treated with anabolic steroids, low doses of estrogen, and/or hGH (1–5). Human GH treatment has not been systematically employed because of the lack of hGH supply. Since the successful synthesis of hGH by recombinant DNA technology (6), several investigators have reported the safety and biological activities of recombinant hGH (r-hGH) in humans (7–10). Recently, larger numbers of Turner patients have commenced treatment with r-hGH (11–15). However, the protocol for hGH treatment differs according to the investigators, and few patients have been treated for more than three years.

We report a multicentre study in Japan, from February 1987 to June 1990, on the clinical effect of three years of r-hGH therapy in 46 patients with Turner’s syndrome. A proportion of these patients are included in subjects reported previously for the effect of one year treatment of hGH (YM-17798) (12, 16).

Patients and methods

Patients and study design

We investigated 46 patients with Turner’s syndrome, aged 5 to 15 years. Informed consent was obtained from each patient and her parents, and the experimental protocol was approved by the Human Subjects Investigation Committee of the participating medical hospitals. Table 1 gives the clinical and laboratory data of these patients. Turner’s syndrome was diagnosed by sex chromosome analysis. Serum hGH responses to provocation testing revealed no classic GH deficiency (peak GH levels ≥7 μg/l). They had not been treated with other growth-accelerating medications. They were prepuber-


A total of 46 patients with Turner’s syndrome were treated for three years with recombinant hGH. Nineteen patients received hGH at a weekly dosage of 0.5 IU·kg-1·week-1, while 27 received 1.0 IU·kg-1·week-1 by daily sc injection. Both treatment groups showed a statistically significant growth increase during treatment. The increase in height over three years’ treatment, as expressed by SDS score (sds) for chronological age, did not differ significantly between patients treated with 0.5 IU and those with 1.0 IU hGH. Seventeen of 22 patients over the age of 14 had exceeded their expected adult height. Plasma IGF-I levels were elevated and no remarkable advances in bone age were observed during the treatment in either treatment group. There were no other significant changes in physical or laboratory examinations. No glucose intolerance was observed. These results indicate that hGH treatment is useful for accelerating growth velocity in patients with Turner’s syndrome. However, further study will be required to find the best treatment dosage.

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<table>
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<th>Weekly dose per kg</th>
<th>0.5 IU</th>
<th>1.0 IU</th>
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<tbody>
<tr>
<td>N</td>
<td>19</td>
<td>27</td>
</tr>
<tr>
<td>Chronological age (years)</td>
<td>10.7 ± 2.4</td>
<td>9.9 ± 2.6</td>
</tr>
<tr>
<td>Bone age (years)</td>
<td>9.4 ± 2.1</td>
<td>8.9 ± 2.5</td>
</tr>
<tr>
<td>Height (sd)</td>
<td>−0.44 ± 1.09</td>
<td>−0.23 ± 1.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>24.7 ± 6.0</td>
<td>23.4 ± 7.4</td>
</tr>
<tr>
<td>Growth rate (cm/year)</td>
<td>3.4 ± 1.1</td>
<td>3.7 ± 1.0</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2710 ± 512</td>
<td>2583 ± 305</td>
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<td>Sex chromosome (persons)</td>
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<tr>
<td>45, X</td>
<td>2</td>
<td>14</td>
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<tr>
<td>Mosaics</td>
<td>16</td>
<td>12</td>
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<tr>
<td>Othersb</td>
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</table>

Table 1. Clinical and laboratory data from 46 patients with Turner’s syndrome treated with r-hGH (YM-17798).

* SD score of untreated Turner growth rate described by Takano et al. (1988).

b Second sex chromosome abnormalities.
tal. Nineteen and 27 patients were treated with 0.5 and 1.0 IU·kg⁻¹·week⁻¹ methionine-free r-hGH, respectively. The weekly dosage was injected SC in six to seven divided doses per week for three years. Vital statistics, height and body weight were checked during the treatment, and blood count, urinalyses, routine laboratory tests, antibody levels to hGH and plasma IGF-1 were measured before and every second month during hGH treatment. Bone age was evaluated both before and every 12 months during treatment. Oral glucose tolerance (1.75 g/kg glucose load) was performed before, 12 and 36 months after the start of treatment.

Growth hormone preparations

The methionine-free r-hGH (YM-17798) used in this study was obtained from Novo-Nordisk A/S, Copenhagen, Denmark. Each vial contained 12 IU hGH (by tibia test compared to a WHO standard), 60–70 mg glycine, 6–8 mg mannitol and 7.5–10 mg Na phosphate.

Assay method

Routine blood and urinary analyses were performed at the participating medical hospital. Plasma IGF-1 was measured with an IGF-1 RIA kit (Nichols Institute, San Juan Capistrano, CA). Anti-hGH antibody was determined by the polyethylene glycol method previously described (17). Bone age was estimated by a specialist using the TW-2 method (20 bones) (18). Height age was calculated using a previously reported cross-sectional growth curve of Turner patients (19). Results are expressed as the mean ± SD. For statistical analysis the parametric tests were used because the data fit normal distribution. Within each treatment group the paired t-test was used to compare treatment results with baseline. Intergroup comparisons were performed with the unpaired t-test. The correlations between various measurements were calculated by linear regression analysis (Pearson’s correlation coefficient). Differences with p < 0.05 were considered significant.

Results

The individual growth rate before and during three years of treatment with hGH was plotted against a previously reported cross-sectional growth curve (19) (Fig. 1). The growth rate before treatment ranged between 1.5 and 6.2 with a mean of 3.6 ± 1.1 cm/year (N = 46). During the first, second and third years of treatment with 0.5 IU·kg⁻¹·week⁻¹, mean growth rates were 6.2 ± 1.2, 4.9 ± 1.1 and 4.0 ± 1.2 cm/year, respectively. With 1.0 IU·kg⁻¹·week⁻¹, mean growth rate increased to 7.4 ± 1.4, 5.1 ± 1.2 and 4.3 ± 1.2 cm/year during these years, respectively. Values observed during the first and second years of treatment were greater than those before treatment. The larger dose increased growth rate to a significantly greater degree only during the first year of treatment. As the natural growth rate in patients with Turner’s syndrome decreases according to age, we calculated growth rate by the ICS of Turner’s growth rate. The mean ICS before and during the first, second and third years of treatment with 0.5 IU·kg⁻¹·week⁻¹ were −0.44 ± 1.09. 3.02 ± 1.27. 1.62 ± 1.03 and 0.89 ± 1.61, respectively. With 1.0 IU·kg⁻¹·week⁻¹, the values were −0.23 ± 1.05. 3.91 ± 1.65. 1.58 ± 1.25 and 1.09 ± 1.20, respectively. The values observed during the three years of treatment were greater than those before treatment. Again, the larger dose increased growth velocity (1CS) significantly only during the first year of treatment. The growth rate and Δgrowth rate before and during the three years of treatment in the various age groups are given in Table 2. The mean ΔICS value was positive during the three years of treatment in the various age groups except those observed during the third year of treatment with 0.5 IU·kg⁻¹·week⁻¹ in patients under the age of 8. There were no correlations between the growth rate increase (ΔICS) for each period on the one hand and the chronological age, bone age, height and plasma IGF-1 values before treatment on the other. However, there was a significant negative correlation with the pretreatment growth rate. The correlation coefficients (r) between growth rate increase (ΔICS) for the first, second and third years and pretreatment growth rate were −0.64, −0.65 and −0.50, in the group treated with 0.5 IU·kg⁻¹·week⁻¹ respectively, and for those treated with 1.0 IU·kg⁻¹·week⁻¹ they were −0.53, −0.74 and −0.64, respectively.

The individual total height increase during three years of treatment is shown as the ICS value for chronological age (Fig. 2). The mean increase of height (ΔICS) were 1.11 ± 0.75 and 1.41 ± 0.59 with 0.5 and 1.0 IU·kg⁻¹·week⁻¹, respectively. These two values are not significantly different. During the three years of treatment, incomplete secondary sex characteristics appeared in four and six patients treated with 0.5 and 1.0 IU·kg⁻¹·week⁻¹ hGH, respectively. As we did not find any difference in growth rate between prepubertal and pubertal children, we combined the data.

As treatment lasted three years, some patients had already exceeded their expected adult height. We calculated the height increase as the current height minus expected adult height at 17 years (Fig. 3). At the age of 14, the height tended to exceed the expected adult height. Over the age of 14, 7 out of 8 and 10 out of 14 patients treated 0.5 IU and 1.0 IU·kg⁻¹·week⁻¹ hGH, respectively, had exceeded their expected adult height after three years of treatment. The actual height of these patients ranged between 132.0 and 146.0, with a mean of 139.4 ± 4.7 cm. However, all children were still below the average height for their age.

During the first, second and third years of treatment with 0.5 IU, mean bone age increased by 1.0 ± 0.6, 1.7 ± 1.0, 2.5 ± 1.0 years, respectively. With 1.0 IU, bone age increased by 0.9 ± 0.6, 1.9 ± 0.9 and 2.9 ± 1.3 years, respectively. In younger patients, bone age was

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Fig. 1. Individual growth rates before (○) and during (●) three years of 0.5 IU·kg⁻¹·week⁻¹ (upper panel) and 1.0 IU·kg⁻¹·week⁻¹ (lower panel) of hGH treatment in 19 and 27 patients with Turner’s syndrome, respectively, plotted against the natural growth rate of Turner’s syndrome patients (mean±SD, ±2 SD) described by Takano et al. (1988). Stars (★) indicate the patients who developed secondary sex characteristics during treatment.
Table 2. Growth rate (sd) and Δgrowth rate before, and during 1, 2 and 3 years' treatment with hGH. (Mean±sd).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>N</th>
<th>Before</th>
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<th>Year 2</th>
<th>Year 3</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
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<tr>
<td>&lt;8</td>
<td>3</td>
<td>0.4±1.6</td>
<td>2.5±1.1</td>
<td>1.3±0.8</td>
<td>-0.2±2.7</td>
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<td>10≤&lt;12</td>
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<td>3.7±1.4</td>
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Fig. 2. Height increase (ΔHSD) during three years of 0.5 IU (left) and 1.0 IU (right)·kg⁻¹·week⁻¹ hGH treatment. Stars indicate the patients who developed secondary sex characteristics during treatment.

greatly advanced, as shown in Fig. 4. However, there was a positive correlation between Δbone age (x) and Δheight age (y); given below are correlations during three years’ treatment with 0.5 IU·kg⁻¹·week⁻¹; y = 0.34x + 1.63, r = 0.65, and with 1.0 IU·kg⁻¹·week⁻¹; y = 0.20x + 2.25, r = 0.51.

Antibodies against hGH were observed in only one patient after one year of treatment. However, this had no effect on her growth rate and they disappeared after 26 months of treatment. No patients had positive antibody titres at the end of the three years of treatment. Plasma IGF-1 levels before, during the first, second and third years of treatment with 0.5 IU and 1.0 IU were 0.7±0.4 and 0.8±0.2, 1.3±0.7 and 2.6±1.5, 1.7±0.7 and 2.9±1.5, 1.6±1.0 and 2.7±1.0 U/ml, respectively. Mean values observed during the three years of treatment with 1.0 IU·kg⁻¹·week⁻¹ hGH were statistically greater than those observed of 0.5 IU. Glucose metabolism was studied before, and at one and three years of hGH treatment by the oral glucose test (Fig. 5). Basal glucose levels increased slightly with the larger dose; however, glucose area under the curve between 0 and 120 min did not change during treatment. Both basal and maximal insulin levels increased in the group treated with 1.0 IU. No patients developed glucose intolerance. Hemoglobin-A1 levels were all within the normal range. There were no significant changes in blood count, urinalysis or routine biochemistry during the treatment.

Discussion

Growth hormone treatment in patients with Turner's syndrome has become widespread since adequate amounts of r-hGH have become available. However, we have not yet obtained the consensus on how properly to
was observed with both doses: the effect, which was greatest during the first year, decreased gradually with the duration of treatment. These results were similar to those reported by Rosenfeld et al. (14, 20) on the longest clinical trial of hGH treatment. Decreased effectiveness of GH treatment according to the duration of treatment might be due to resistance of the tissues for GH and/or IGF-1. However, the mechanism of this phenomenon is not clear at present and further study will be required to solve this question. Compared with the dose, the larger dose increased growth rate significantly only during the first year of treatment. Furthermore, the actual height increase did not differ significantly during the third year of treatment. From these observations, we might conclude that the dosage of 0.5 IU·kg⁻¹·week⁻¹ had the same effect during the three years of treatment. However, the numbers of patients are small in this study and further investigations will be required to confirm this conclusion.

In this study we observed that the effect of treatment is greater in patients who had lower growth rate before treatment. Although these patients showed a serum GH increase above 7 μg/l by the GH provocation test, the daily GH secretion might be reduced.

Adverse events in our study were very few. Glucose intolerance did not occur in any patient, although basal and maximal insulin levels after oral glucose administration increased slightly. Thus, long-term hGH treatment demands careful attention. Bone age did not advance beyond the changes of chronological age. The greatly increased bone age observed in younger patients seems to be not much of a problem since height age increased more than bone age. This methionine-free hGH preparation is infrequently antigenic. At the end of one year treatment antibody titres were observed in 5 of 80 patients (6.3%) (16), an incidence similar to the 6.1% observed in the treatment of pituitary dwarfs with the

treat patients with hGH, such as the time for initiating treatment, the dosage of hGH and so on. In this study, we report on the effects of two doses of hGH treatment without any combination therapy. Growth acceleration

Fig. 3. Height increase in Turner's syndrome patients treated with 0.5 IU (○) and 1.0 IU·kg⁻¹·week⁻¹ (●) hGH. Current height minus expected adult height is shown. Stars indicate patients with secondary sex characteristics.

Fig. 4. Bone age increase (ΔBA) during three years of 0.5 IU (left) and 1.0 IU (right)·kg⁻¹·week⁻¹ hGH treatment. Stars indicate the patients who developed the secondary sex characteristics during treatment.
same preparation (10). The antibodies tend to disappear with continuing therapy.

Many investigators are now treating Turner patients and each clinical group has its own protocol; for example, treatment with hGH only or in combination with anabolic steroids and/or estrogen. Rosenfeld et al. reported the longest clinical trial of hGH treatment (14, 20). Their treatment of 67 patients started in 1983, their first study comprising four treatment groups—a control group (no treatment), an oxandrolone (0.125 mg·kg⁻¹·day⁻¹, po) group, an hGH (0.375 mg·(0.75 IU)·kg⁻¹·week⁻¹) treated group and a combination of an hGH and oxandrolone group. In the next study, the hGH-treated group remained unchanged while the other three groups were treated with a combination of hGH and a half-dosage of oxandrolone. In the GH-treated group (N = 17), growth rates before and during the first, second and third years were 4.5 ± 0.8, 6.6 ± 1.2, 5.4 ± 1.1 and 4.6 ± 1.4 cm/year, respectively. These results over three years were similar to ours. The growth velocity was greatest during the first year of treatment and decreased gradually in the ensuring years. Since this decrease occurs at all ages, presently unknown factors other than bone maturation must be involved in the explanation of this phenomenon. After five years of treatment, 11 of the 17 patients had already exceeded their expected adult height. The increase in growth rates was better with combination therapy than with GH alone. Several patients had already reached the mean height of normal girls. Bone age increased 4.4 ± 1.9 years during the five years of therapy. Thus, the combination therapy of hGH with a small dose of oxandrolone shows great promise in increasing the height of Turner girls to normal.

Estrogen therapy in combination with hGH administration has also been reported (15, 21, 22). Combined estrogen had no additional effect on height increase, but even very low doses of estrogen induced breast development at an early age and accelerated bone maturation. Similar results have also been reported with combination therapy using the three agents hGH (0.1 IU·kg⁻¹·d⁻¹), oxandrolone (0.05 mg·kg⁻¹·d⁻¹) and estrogen (100 mg·kg⁻¹·d⁻¹) (23). These studies did not show any effect of estrogen on height increase, but did show advance bone age. The use of estrogen with hGH therapy is therefore not recommended before the age of 13–14.

The mechanism of the effect of hGH treatment in Turner patients is not clear. We and others have observed that 24 h GH secretion, as determined by 20 min blood sampling, was relatively low in prepubertal and adolescent patients (24, 25). Therefore, additional hGH administration may be effective in inducing height increases. On the other hand, this may be partially based on a mechanism similar to that of gigantism in prepubertal children with GH-producing tumors. According to the data of 4–5 years' treatment, we are confident that GH therapy in Turner's patients is a promising way to increase final adult height. However, further studies will be required to find the ideal age for the initiation of GH therapy, the best dosage, regimen use of anabolic steroids, and finally the optimal time and manner of sex steroid replacement.

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References


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