Effects of hGRF Treatment of A Patient with hGRF Deficiency

KAZUE TAKANO, NAOMI HIZUKA, IZUMI TANAKA AND KAZUO SHIZUME

Institute of Clinical Endocrinology, Tokyo Women’s Medical College, 10 Ichigaya Kawada-cho, Shinjuku-ku, Tokyo 162

Abstract

A 12-year old girl was admitted to our hospital for evaluation of her short stature. Her height was below −3.5 SD of the mean height for her age. She was diagnosed as having craniopharyngioma and treated surgically. Thereafter she was treated with thyroxine and hydrocortisone. One and a half years later, she revisited our hospital for treatment of short stature. Her plasma GH did not respond to insulin-induced hypoglycemia but increased after hGRF-44 administration, indicating hGRF deficiency. hGRF was therefore administered at a dosage of 100 µg twice a day subcutaneously for three months. Her height increased 1.6 cm during treatment, which corresponded to a height increase of 6.4 cm/year.

These findings indicate that hGRF treatment stimulates height increase in patients with GRF deficiency. For complete evaluation of hGRF therapy, further studies will be required.

Growth hormone releasing factors (GRFs) have been isolated and characterized from pancreatic tumors in patients with acromegaly (Guillemin et al., 1982; Esch et al., 1982; Rivier et al., 1982). Ling et al., (1984) isolated and characterized human hypothalamic GRF from human pituitary stalk median eminence extract and reported that its structure is identical to that of the 44-amino-acid GH releasing peptide isolated from the tumors. Thus we use the term hGRF to designate the human growth hormone releasing factor, either tumorous or hypothalamic. Using synthetic replicates of these human pancreatic GRFs, it has been demonstrated that the majority of patients with GH deficiency have GH responses to a single iv bolus and/or repeated administrations of the peptide by either im, sc or iv infusion (Borges et al., 1983; Grossman et al., 1983; Takano et al., 1984; Hizuka et al., 1984). These patients are classified as hGRF deficient. When a patient is so diagnosed, hGRF administration may be a suitable replacement therapy. However, only two cases with GRF deficiency were reported concerning the GRF treatment (Thorner et al., 1985).

In the present study, we reported the effects of hGRF administration for three months in a patient with hGRF deficiency.
Material and Methods

Peptide preparation
Human growth hormone releasing factor (hGRF-44) was kindly provided by Drs. R. Guillemin and N. Ling that was synthesized by the solid phase method (1982). One hundred milligrams of the peptide was dissolved in 100 ml distilled water containing 1 mM ascorbic acid and sterilized by filtration through a 0.22 µm filter (Millipore Corp.). One hundred microliter aliquots of the peptide solution (100 µg) were prepared in sterile vials, lyophilized, and stored at -20°C. A vial was diluted with 1 ml physiological saline immediately before injection.

Test protocol
The subject was fasted overnight and remained recumbent throughout the study. The insulin induced hypoglycemic test (ITT) was performed using iv 0.05 U/kg BW doses of regular insulin to evaluate GH and cortisol secretion. Glucagon-propranolol administration was performed with 2 and 10 mg, respectively, to evaluate GH secretion. Human GRF-44 was administered in a dose of 50 µg intravenously to evaluate the pituitary function of GH secretion before and during hGRF treatment. Plasma LH, FSH, TSH and PRL responses were determined by combined iv administration of 100 µg GnRH and 500 µg TRH. Blood samples were taken at 0, 15, 30, 60, 90, 120, 150 and 180 min after loading, as indicated in Table 1. For long term hGRF treatment, hGRF at 100 µg twice a day was injected subcutaneously. Blood samples for the determination of somatomedin C during therapy were obtained 16–20 hours after injection. Height was measured every month at the same time of day by a single observer. Informed consent was obtained from the patient and her parents and the experimental protocol was approved by the Human Subjects Investigation Committee of our department.

Radioimmunoassays of hormones
Plasma hGH was measured with a commercial radioimmunoassay kit (Eiken Immunochemical Laboratory, Tokyo, Japan). The inter- and intra-assay coefficients of variation were 4.1% and 12.5%, respectively. The normal adult range (from 2 SD below to 2 SD above the mean) is 0.38–1.59 U/ml. Other hormones were assayed with commercially available RIA kits (Eiken Immunochemical Laboratory). The inter- and intra-assay variations of these RIA kits were similar to those of the GH RIA kit. Antibody to hGRF was determined by incubation with 125I-hGRF-44 for 48 hours and antibody-bound tracer was separated from free tracer by precipitation with PEG (Shibasaki et al., 1984).

Case Report
A 12-year-old girl was admitted to our hospital in December of 1982 for investigation of short stature. She was born on the 1st of November, 1970 at full term and weighed 3,080 g. She was fine until she went to kindergarten, when it was first noticed that her growth was retarded. However, as all other physical and mental development was normal, her parents did not consult any doctors until she was 12 years old (Fig. 1). Her linear growth rate over the previous three years has been 2.2, 2.6 and 3.1 cm per year, respectively, for a mean of 2.6 cm per year.

When she was admitted to the hospital her height and body weight were 125.1 cm and 23 kg, which were below -3.5 SD and -2.3 SD of the mean values for her age. Her bone age was 8 years. Except for her short stature, her physical appearance was unremarkable. Her urine, biochemical, blood and visual examinations were normal. The pituitary functions were normal as shown in Table 1. Tomography of the sella disclosed signs of calcified tumor in the suprasellar region. The brain CT scan disclosed an oval-shaped low density area in the midportion of the intrasellar region to suprasellar region with calcification of the wall and moderate dilatation of the lateral and third ventricles (Fig. 2).
She was operated on 26th of January, 1983, and histological findings led to a diagnosis of craniopharyngioma. She had a ventriculo-peritoneal shunt after the operation. The pituitary function tests after surgery disclosed a lack of GH, ACTH, TSH and GnH secretions after insulin induced hypoglycemia, and glucagon-propa-nolol, TRH, and LH-RH provocative tests, respectively. She was treated with daily dosages of 100 µg of T₄, 30 mg of hydrocortisone and anticonvulsant by the Department of Neurosurgery. Three and four months later she was operated on for peritonitis and for abdominal ileus in March and April, respectively. She was operated on again to create a ventricle-cardio shunt to depress the cranial pressure in June of 1983. The maximal GH response to hGRF performed 11 months (in December 1983) after surgery was 28.3 ng/ml (Fig. 3).

Nineteen months after surgery, she visited our clinic for treatment of her growth retardation. The pituitary function test disclosed impaired GH, ACTH, TSH and GnH secretions after respective provocative tests (Table 1). Negative responses of LH, FSH and TSH to LH-RH and TRH tests were considered to be due to the replacement

---

**Table 1. Basal and stimulated hormone levels before and after operation**

<table>
<thead>
<tr>
<th>Before operation (min)</th>
<th>After operation (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 15 30 60 90 120</td>
<td>0 15 30 60 90 120</td>
</tr>
<tr>
<td>ITT GH (ng/ml)</td>
<td>2.2  7.9 11.0 10.6</td>
</tr>
<tr>
<td>F (µg/dl)</td>
<td>27.6 27.3 31.3 26.8</td>
</tr>
<tr>
<td>BG (mg/dl)</td>
<td>91  61 68</td>
</tr>
<tr>
<td>LH-RH LH (mIU/ml)</td>
<td>19.6 32.9 32.8 32.9 32.4</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>8.1 18.8 17.4 20.5 24.0</td>
</tr>
<tr>
<td>TRH TSH (µU/ml)</td>
<td>3.5 23.3 27.5 30.1 17.8</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>15.4 65.0 53.9 41.0 27.2</td>
</tr>
<tr>
<td>GP GH (ng/ml)</td>
<td>1.7  1.7 8.9</td>
</tr>
</tbody>
</table>

basal level

T₄ 184 ng/dl, T₃ 9.9 µg/dl
fT₄ 1.22 ng/dl, F 1.3 µg/dl

T₃ 120 ng/dl, T₂ 7.2 µg/dl
fT₄ 0.94 ng/dl, F 29.6 µg/dl
Somatomedin C 0.22 U/ml

* 150 min after GP administration  ** 180 min after GP administration
therapy of T₄ and high dosage of hydrocortisone. The plasma somatomedin C level was low with a value of 0.22 U/ml. However, hGRF stimulated GH secretion from a basal level of 1.9 ng/ml to a maximal level of 18.0 ng/ml. Her growth rate after surgery was 1.5 cm/year.

With the consent of the patient and her parents, hGRF-44 therapy was started in August of 1984 and continued for 3 months. The doses of 100 µg twice a day were injected subcutaneously. Plasma GH levels after sc administration increased from the basal level of 1.6 ng/ml to the maximal level of 5.1 ng/ml. During the 3 months of treatment together with 100 µg thyroxine and 5 mg hydrocortisone per day, her height increased from 128.3 to 129.9 cm, which was 1.6 cm/3 months and equivalent to 6.4 cm/year. The plasma GH responsiveness to hGRF did not change significantly during the 3 months of treatment (Fig. 3). Plasma somatomedin C did not change significantly (Table 2). No side effects were seen in physical or laboratory examinations. Antibodies to hGRF had not developed by the end of the therapy.

After hGRF treatment was ceased, she

Fig. 2. CT scanning of the brain;
Oval shaped low density noted in midportion of intrasellar region to suprasellar region with calcification of its wall.

Fig. 3. Plasma GH responses to 50 µg iv bolus administration of hGRF-44 before (○—○) and during (●—●) three months of hGRF-44 treatment. Months shown in Fig. indicate months after operation for craniopharyngioma.
received treatment with 2 mg of hGH (Crescormon®) three times a week. She is now on 3 months treatment and her height has increased from 129.9 to 130.3 cm, which is 0.4 cm/3 months. Plasma Somatomedin C has increased to the normal value of 0.82 U/ml during the hGH treatment.

Table 2. Plasma Somatomedin C levels (U/ml) during hGRF and hGH treatment for 3 months.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>1 M</th>
<th>2 M</th>
<th>3 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>hGRF</td>
<td>0.22</td>
<td>—</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td>hGH</td>
<td>0.27</td>
<td>0.40</td>
<td>0.73</td>
<td>0.82</td>
</tr>
</tbody>
</table>

**Discussion**

We have reported here a patient with GRF deficiency treated with daily subcutaneous administration of hGRF for three months. We diagnosed this patient to have GRF deficiency. Because she did not show any plasma GH increase in response to either insulin induced hypoglycemia or glucagon-propranolol test, however she showed a marked GH increase to hGRF-44, indicating hypothalamic GRF deficiency. Other pituitary hormones such as TSH, ACTH, LH and FSH did not respond to their provocative test performed at 19 months after surgery. These negative responses were considered to be due to T₄ and high dosage of hydrocortisone (30 mg/d) replacement therapy. Recently, we have again tested her pituitary function and found that she showed a plasma GH increase (peak value, 13.1 ng/ml) in response to hGRF-44 and a plasma cortisol increase (peak value, 7.2 μg/dl) in response to CRF administration. However, her gonadotropins did not respond to LH-RH administration. Repeated administration of LH-RH might be necessary to obtain increased LH and FSH responses. Her plasma PRL levels increased from 18.5 to 27.7 ng/ml in response to TRH administration. Considering these results together, her pituitary disorder was indicated to be due to hypothalamic dysfunction.

We selected the daily subcutaneous route of hGRF administration because, in preliminary studies, we had observed that a subcutaneous administration of hGRF stimulated growth hormone secretion in patients with GH deficiency (Takano et al., 1985). Her plasma GH levels increased to the maximal level of 5.1 ng/ml in response to sc administration of 100 μg hGRF. Although plasma somatomedin C levels did not rise to the normal range, her height increased 1.6 cm during the three months of treatment with hGRF, which corresponded to a growth rate of 6.4 cm per year. This value was four times more than that before treatment, which was 1.5 cm per year.

Thorner et al. (1985) reported the effectiveness of pulsatile therapy with hGRF-40 in two patients with GH deficiency caused by hypothalamic disorders. They were given 1–3 μg/kg BW of hGRF-40 subcutaneously over one minute every three hours by infusion pump for 28 weeks. Both patients had accelerated growth velocities of 3.8 and 7.4 cm during therapy, which corresponded to 7.1 and 13.7 cm per year. These values were greater than those before treatment (4.6 cm/year and 2.1 cm/year, respectively). During treatment, the former patient did not show any plasma somatomedin C rise; however, the latter showed a somatomedin C increase from 0.09 to a maximum of 0.70 U/ml after three months of therapy. Comparing our case to these two reported ones, ours resembles the former one with respect to growth rate and to the plasma somatomedin C level. Concerning the relationship between the height increase and plasma somatomedin C level, plasma somatomedin C does not always seem to be increased. Other growth factors might play a role in the height increase. However, details of the mechanism are not yet clear.

These results indicate that hGRF ad-
ministration has an effect on growth stimulation in human subjects. It is too early to speculate on the advantages of hGRF over GH in the treatment of these children. The patterns of administration, subcutaneously twice a day or pulsatile infusion, seem more troublesome than hGH treatment. GH therapy is widely used in treating patients with GH deficiency by im injection of three to four times a week. More experience is required to evaluate of hGRF therapy with respect to dose, frequency of administration, and a strong, long acting analog of hGRF.

Acknowledgement

This research was partly supported by Grants in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 58571012, No. 58440084 and No. 59770840), the Intractable Diseases Division, Public Health Bureau, Ministry of Health and Welfare of Japan, and the Foundation for Growth Science and Medical Research Foundation of Japan.

References


