Short-term Study of Biosynthesized hGH in Man

KAZUE TAKANO, NAOMI HIZUKA, KAZUO SHIZUME, KUMIKO ASAKAWA AND MEGUMI KOGAWA

Department of Medicine, Tokyo Women's Medical College
Tokyo 162

Abstract

Daily injection of 8IU of methionyl human growth hormone (met-hGH), produced by recombinant DNA technology, was performed in healthy young volunteers. Daily injections for four days did not cause any significant change in the results of physical examination, electrocardiogram, blood count or urinalysis.

Non-esterified fatty acid levels increased significantly at 4 hours after the first injection of met-hGH in a manner similar to pituitary human growth hormone (pit-hGH). Both hGHS caused a significant increase in serum somatomedin A during the four daily injections. Plasma hGH reached peak levels at 3-4 hours after im injection of met-hGH and 4-6 hours after pit-hGH. The levels were 19.5±1.6 and 21.3±2.8 ng/ml, respectively. After iv one bolus administration of 4IU hGH to two volunteers, plasma hGH reached peak levels at 5 minutes at mean levels of 292 ng/ml after met-hGH and 293 ng/ml after pit-hGH. The half life of the two hGHS was 17.2 and 17.1 minutes, respectively, according to the disappearance curve measured after iv administration.

Since the success of hGH synthesis by recombinant DNA technology (Goeddel et al., 1979), several investigators have reported tests of methionyl-hGH (met-hGH) for its properties and biological activities 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). In this paper, we have investigated the acute effect of met-hGH in eight healthy male volunteers and compared them with those of pituitary human growth hormone. We have also studied the disappearance rate of hGH after iv administration to two volunteers.

Materials and Methods

Growth hormone preparations
Synthetic methionyl human growth hormone (met-hGH) (No. 81651) was obtained from AB KABI, Stockholm, Sweden. Human pituitary growth hormone (pit-hGH) used in this study was Crescormon® (KABI, Sweden). Each vial contained 4IU hGH (by RIA), 40 mg glycine, and 1 mg Na-phosphate. For daily im injection, 2 vials were dissolved in 2ml of distilled water before injection. For iv injection, 1 vial was used and dissolved in 2ml of distilled water.

Study design
Eight healthy male volunteers aged between 20 and 21 years were divided into two groups. All volunteers were within 10% of ideal body weight with a mean of 66.2±3.0 kg. Each group consisted of four volunteers who received daily im injections.
of either 8IU met-hGH or pit-hGH for four days without awareness. On the 10th day after the last injection, pit-hGH or met-hGH was injected into the other group in a similar manner. The injections of hGH were given daily at 8:00 am. During the study, vital signs were checked by a physician. Blood count, urinalysis, 12-lead electrocardiogram, routine blood chemistry, and serum T3, T4, and TSH measurements were performed before and after the four daily injections of hGH. Serum somatomedin and insulin were assessed daily 24 hours after the previous injection. After the first injection, plasma hGH was determined at 0, 1, 2, 3, 4, 6, 10 and 24 hours, and non-esterified fatty acid was measured at 0 and 4 hours.

Intravenous hGH was administered to two other volunteers. They received 4IU met-hGH and pit-hGH with a one week interval between them. Plasma hGH, insulin, glucose and NEFA were measured over two hours at the times indicated in the results.

**Assay methods**

Plasma GH was determined by double antibody RIA, a gift from the National Pituitary Agency, NIADDK (hGH: AFP-4793B, Antibody: AFP-977201-33). Serum insulin was measured by double-antibody technique using the kit provided by Eiken Chemical Co., Ltd. (Tokyo, Japan). Serum somatomedin was determined by the radioreceptor assay for somatomedin A described previously (Takano et al. 1976). Anti-hGH antibody was determined by the polyethylene glycol method (Besbuquois and Aurbach 1971). Blood glucose, cholesterol, non-esterified fatty acid, and other substances in blood were measured by standard automated techniques in the laboratory of Tokyo Women's Medical School Hospital. The radioreceptor assays for hGH were performed by the method utilizing IM-9 cells and pregnant rabbit liver membranes described earlier (Hizuka et al. 1982).

**Statistical analysis**

Student's t-test and paired t-test were used for statistical analysis. Period effect (time trend) was statistically analyzed according to the method described by Hills and Armitage (1979).

**Results**

In the RIA, the dose-response curves for met-hGH and pit-hGH paralleled the pituitary hGH standard from the National Pituitary Agency (Fig. 1). In the RRAs using IM-9 cells or pregnant rabbit liver membrane, met-hGH and pit-hGH behaved in the same manner as pituitary hGH standard (data not shown). When the ratio of radioreceptorassayable hGH to radioimmunoassayable hGH (RRA/RIA) was calculated, the ratio for met-hGH was 1.0 which was identical to the pituitary hGH standard. When met-hGH was gel filtered on Sephadex
G-100, the immunoreactive hGH eluted primarily as a simple peak (data not shown).

Changes in serum hGH concentration after im injection of both preparations are shown in Figure 2. The levels of hGH rose and reached peak levels between 3 and 4 hours after met-hGH and between 4 and 6 hours after pit-hGH injections. The mean peak values of both hGHs were similar, being 19.5±1.6 and 21.3±2.8 ng/ml, respectively. There was a significant difference between the hGH levels 2 and 3 hours after injection. However, the integral values calculated from the both curves did not differ significantly from each other.

The acute lipolytic effect of hGH was examined by measuring the non-esterified fatty acid (NEFA) before and 4 hours after the injections. As shown in Figure 3, NEFA significantly increased from 0.35±0.06 to 0.97±0.10 mEq/L after met-hGH and from 0.36±0.05 to 0.89±0.05 mEq/L after pit-hGH (p<0.001). The chronic somatotrophic effect on somatomedin generation was studied by measuring serum somatomedin A 24 hours after the four daily injections (Fig. 4). Both hGHs caused a slight but significant increase in radio-

![Fig. 2. Changes in plasma hGH level after im injections of 8IU of met-hGH (●●) and pit-hGH (○○). Vertical lines indicate the mean ± SEM.](image)

![Fig. 3. Changes in serum NEFA before and 4h after im injections of 8IU of met-hGH (left) and pit-hGH (right). Vertical lines indicate the mean ± SEM.](image)
Fig. 4. Changes in serum somatomedin A during daily injections of 8 IU of met-hGH (■) and pit-hGH (□) for four days. Bars and brackets indicate the mean ± SEM. The difference from day 0 is significant at p<0.05 (*) and p<0.001 (**).

Fig. 5. Changes in plasma hGH level after iv injections of 4 IU of met-hGH (●-●) and pit-hGH (○-○) to two volunteers. Plasma hGH was determined at 0, 5, 10, 15, 20, 30, 45, 60 and 120 minutes after injection.

Table 1. The changes in blood count, blood chemistry and other values before and 24 h after the last of 4 daily injections of hGH. (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>met-hGH Before</th>
<th>met-hGH After</th>
<th>pit-hGH Before</th>
<th>pit-hGH After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocyte (×10⁹/mm³)</td>
<td>6300±382</td>
<td>5700±463</td>
<td>6338±692</td>
<td>5413±535*</td>
</tr>
<tr>
<td>Erythrocyte (×10¹²/mm³)</td>
<td>494±11</td>
<td>475±13</td>
<td>498±14</td>
<td>479±14***</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>15.3±0.3</td>
<td>14.6±0.4</td>
<td>15.4±0.4</td>
<td>14.8±0.4**</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>46.1±0.8</td>
<td>44.4±1.1</td>
<td>46.6±1.2</td>
<td>44.8±1.0**</td>
</tr>
<tr>
<td>Platelet (×10⁹/mm³)</td>
<td>24.0±1.2</td>
<td>21.9±1.1*</td>
<td>24.3±1.4</td>
<td>24.0±1.3</td>
</tr>
<tr>
<td>T₄ (µg/dl)</td>
<td>8.3±0.4</td>
<td>7.4±0.3*</td>
<td>8.4±0.5</td>
<td>7.5±0.3*</td>
</tr>
<tr>
<td>TSH (µU/ml)</td>
<td>2.1±0.6</td>
<td>1.5±0.4*</td>
<td>2.1±0.4</td>
<td>1.7±0.5</td>
</tr>
<tr>
<td>Total protein (d/dl)</td>
<td>7.0±0.1</td>
<td>6.9±0.1</td>
<td>7.2±0.1</td>
<td>6.9±0.1**</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>14.8±0.9</td>
<td>10.8±0.8***</td>
<td>14.0±0.9</td>
<td>12.0±0.5</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.3±0.0</td>
<td>1.2±0.0**</td>
<td>1.25±0.0</td>
<td>1.2±0.0</td>
</tr>
<tr>
<td>LDH (mU/ml)</td>
<td>159±4</td>
<td>149±6</td>
<td>176±11</td>
<td>145±4*</td>
</tr>
<tr>
<td>Al-P (Unit)</td>
<td>5.8±0.5</td>
<td>5.6±0.7</td>
<td>6.0±0.7</td>
<td>5.5±0.7*</td>
</tr>
<tr>
<td>Cholesterol (mg/ml)</td>
<td>148±8.0</td>
<td>127±5.2**</td>
<td>152±8.2</td>
<td>136±5.3*</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.85±0.10</td>
<td>0.61±0.05**</td>
<td>0.70±0.07</td>
<td>0.65±0.07</td>
</tr>
<tr>
<td>A/G</td>
<td>1.68±0.09</td>
<td>1.69±0.06</td>
<td>1.66±0.05</td>
<td>1.76±0.06*</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>96±8.4</td>
<td>88±9.2*</td>
<td>96±9.9</td>
<td>91.5±9.1</td>
</tr>
</tbody>
</table>

The difference from the day before is significant at p<0.05 (*), p<0.01 (**) and p<0.001 (**). The following did not change significantly after either injection: GOT, GPT, γ-GTP, Ca, P, Na, K, Triglyceride, NEFA, Albumin, Globulin, β₂-microglobulin, IgA, IgG, TIBC, UIBC, Fe, T₃, glucose, CRP.
receptor-assayable somatomedin A after a single injection and maintained the increased levels during the injections. There were no significant differences between met-hGH and pit-hGH in their ability to increase serum NEFA and somatomedin A. Serum insulin concentrations did not change significantly during hGH injections.

After one intravenous bolus administration of 4IU hGH to two volunteers, the serum concentrations of hGH increased rapidly and reached 236 and 348 ng/ml 5 minutes after met-hGH and 278 and 308 ng/ml 5 minutes after pit-hGH injection, respectively (Fig. 5). The half lives of the two hGHs in the two volunteers did not differ greatly and the values were 16.6 and 17.7, and 16.9 and 17.2 minutes, respectively, when calculated from the disappearance curve between 10 and 45 minutes after hGH administrations. Serum insulin, NEFA and blood glucose did not change significantly when they were measured at 15, 30, 60 and 120 minutes after hGH administration.

During the short-term administration of the two hGHs, there were no pyrogenic effects, and no significant changes in physical examination, electrocardiogram and urinalysis results. The results of the paired t-test revealed some changes in blood count, routine blood chemistries, T₄, and TSH levels, as shown in Table 1; however, the differences were within the normal ranges. Anti-hGH antibody did not appear in the serum obtained before, immediately after the 4 daily injections, or 30 days after the first injection of either hGH. There is no periodic effect on the parameters which we examined in this study.

Discussion

The human growth hormone produced in E. coli has an extra methionine residue at the aminoterminal end of the mature hGH. We studied its properties and confirmed that methionyl hGH (met-hGH) has characteristics with respect to gel filtration, RIA, and RRA indistinguishable from those of pit-hGH, which were reported by Hizuka et al. (1982).

The short term injection of 8IU met-hGH did not cause any side effects such as fever, liver dysfunction, or the appearance of anti-hGH antibody. Rosenfeld et al. (1982) reported that met-hGH increased the plasma glucose and insulin levels after 16 IU daily im injections to normal volunteers. We did not obtain similar results, probably being due to the amount of met-hGH used in our study, which was half of theirs. Hintz et al. (1982) reported decreases in blood urea nitrogen and cholesterol, and an increase in serum triglycerides measured 18 hours after the last of 4 daily injections. We observed similar results when blood samples were measured 24 hours after the last of the 4 daily injections. We also observed the metabolic effect of met-hGH increasing non-esterified fatty acid and somatomedin A. These effects were indistinguishable from those obtained with pit-hGH.

After im injection of hGH, the peak level seems to appear earlier with met-hGH than pit-hGH. This result is similar to that reported by Hintz et al. (1982). As the disappearance rate of the two hGHs did not differ significantly, these results suggest that met-hGH absorbs faster than pit-hGH.

We conclude that short-term administration of met-hGH did not show any side effects and met-hGH had a biological effect similar to that of pit-hGH. The long-term effects of met-hGH on growth in patients with growth hormone deficiency will be studied.

Acknowledgements

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References


