Subcutaneous degradation of biosynthetic human growth hormone in growth hormone deficient patients

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Abstract. The aim of the present study was to look further into the question of local degradation of sc injected human GH in GH deficient patients. A comparison was made of serum GH levels after constant iv and sc infusion of the same amount of GH (33 ng · kg⁻¹ · min⁻¹) in the same 9 GH deficient patients. A 3-h lag period was interposed between the iv and the sc infusion. Iv infusion was continued for 3 h. All 9 subjects subsequently received sc infusion for 19 h and five of them continued for additionally 24 h. The mean steady state serum GH level in the nine patients was 23.1 ± 5.1 µg/l after iv and 6.8 ± µg/l after sc administration (P < 0.01). Extension of the sc infusion period in 4 of the subjects did not significantly alter the serum GH level (P > 0.15), implying that a steady state was reached. The GH in the infusion system was stable throughout a 24-h period. We therefore conclude that sc injected GH is degraded locally to a substantial extent.

GH replacement therapy has traditionally been administered as im injections twice or thrice weekly.

The im route was originally chosen because sc injections had induced local atrophy and was suspected of inducing higher levels of GH antibodies (Underwood et al. 1973). Recently, several investigators used the sc route without adverse effects (Christiansen et al. 1983; Wilson et al. 1985). Indeed, many pediatricians now recommend the use of daily sc injections of GH (Wilson et al. 1985; Kastrup et al. 1983; Albertsson-Wikland et al. 1986; Hermanussen et al. 1985).

In a recent study comparing pituitary and biosynthetic human GH we found a significantly reduced bioavailability of both GH preparations after sc compared with im injections as judged by the 24-h serum GH profiles (Jørgensen et al. 1987). Sc degradation of the injected GH seemed the most reasonable explanation, but very slow local accumulation could not be ruled out.

In the present study we looked further into this problem by comparing serum GH levels after constant iv and sc GH infusion during steady state.

Patients and Methods

Patients
Nine GH deficient patients were studied. Clinical data are listed in Table 1. GH deficiency was defined as a peak GH response below 5 µg/l to at least 1 stimulation test, namely arginine and/or external heating (Christensen et al. 1984). None of the patients developed GH antibodies in the period observed.

Design
The study was designed to obtain steady state serum values of GH after constant iv and sc infusion of a fixed amount of GH in the same subjects on two consecutive occasions. The evening before the start of the study no
GH was given, whereas the other medications listed in Table 1 were continued. After an overnight fast, an iv catheter was placed in an antecubital vein in each arm. Three basal blood samples were drawn during the following hour with the patients at rest in the supine position. Iv infusion of GH was then commenced lasting for 3 h. This was followed by a 3-h interval, after which sc infusion was started. All 9 subjects received sc infusion for the following 19 h and 4 of the subjects (No. 5–9) continued for a further 24-h period. During iv infusion, blood samples were drawn every 15 min; during sc infusion blood was sampled every 30 min for the last 3 h of each infusion period. The patients were non-fasting and moderate physical activity was allowed.

The infusion rate of GH was 33 ng·kg⁻¹·min⁻¹, except in subject No. 9 who received 55 ng·kg⁻¹·min⁻¹. The GH preparation (Norditropin®️, Nordisk Gentofte, Denmark) consisting of two 12-IU vials of biosynthetic human GH (specific activity = 3 IU/mg) was dissolved in sterile isotonic NaCl to a total volume of 600 ml. The GH solution was infused by means of an iv infusion set and pump (Terufusion STC-503, Rødovre, Denmark).

One of the antecubital vein catheters (Venflon) was used for iv infusion; for sc infusion a 19-mm 25 G cannula (Terumo) was placed in the abdominal sc tissue.

Control of GH stability
The stability of the dissolved GH was tested by measuring the GH concentration in the infusion system at room temperature for 24 h during which samples were automatically collected at 30-min intervals.

**Table 1.**
Clinical data on the participating GH-deficient patients at the onset of the study.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age years</th>
<th>Height cm</th>
<th>Weight kg</th>
<th>Diagnosis</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>21</td>
<td>173.0</td>
<td>68.2</td>
<td>Germinoma</td>
<td>Cortisone, levotyroxine, desmopressin, testosterone</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>29</td>
<td>178.0</td>
<td>99.7</td>
<td>Germinoma</td>
<td>Cortisone, levotyroxine, desmopressin, testosterone</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>15</td>
<td>163.0</td>
<td>55.0</td>
<td>Cromophobe adenoma</td>
<td>Human growth hormone</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>12</td>
<td>156.0</td>
<td>32.1</td>
<td>Isolated GH deficiency</td>
<td>Human growth hormone</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>16</td>
<td>157.0</td>
<td>52.1</td>
<td>Isolated GH deficiency</td>
<td>Human growth hormone</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>22</td>
<td>133.0</td>
<td>50.0</td>
<td>Isolated GH deficiency</td>
<td>Nil</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>22</td>
<td>174.0</td>
<td>60.2</td>
<td>Isolated GH deficiency</td>
<td>Nil</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>21</td>
<td>165.0</td>
<td>57.0</td>
<td>Cystis epidermoides</td>
<td>Cortisone, levotyroxine, desmopressin, testosterone</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>23</td>
<td>150.0</td>
<td>51.0</td>
<td>Isolated GH deficiency</td>
<td>Human growth hormone</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>20.1</td>
<td>161.0</td>
<td>58.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>± SEM</td>
<td></td>
<td>1.7</td>
<td>4.5</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analyses and statistics
Serum GH was measured by RIA as previously described (Ørskov et al. 1968). Intra-assay coefficient of variation was 6.2%. Standard hGH preparation was obtained from Nordisk Gentofte, Denmark (Bio-synthetic hGH, 1 mg = 3.0 IU). All samples from one person were run in the same assay.

Student’s t-test was used for statistical calculations. All results are expressed as mean ± SEM.

Results
The individual serum GH values are presented in Table 2.

The mean serum GH levels during the last 45 min of the 3-h iv infusion and the last 3 h of the 19-h sc infusion in all 9 subjects are shown in Fig. 1. The mean GH level during iv infusion was 23.1 ± 5.1 μg/l and during sc infusion 6.8 ± 1.6 μg/l. The difference between these 2 serum GH levels was highly significant (P = 0.002).

Patients No. 5–9 continued on sc infusion for an additional 24-h period. Their mean iv value was 25.6 ± 6.3. The mean value during the 16th–19th h of sc infusion was 7.4 ± 2.4 μg/l and 8.7 ± 2.6 μg/l during the 40th–43rd h of sc infusion.
Table 2.
Individual serum GH values in all 9 patients during the study. The basal value in the table is the mean of 3 samples drawn 1 h prior to infusion.

| Patient No. | Serum GH levels (µg/l) |  |
|-------------|------------------------|--|---|---|---|---|---|---|---|---|---|
|             | Basal                  | iv infusion | sc infusion (19 h) | sc infusion (43 h) |  |
| 1           | 0.5                    | 25 26 22   | 8.8 9.0 11 9.4 11 7.0 | - - - - - - - - - |
| 2           | 0.5                    | 14 15 15   | 4.0 4.6 3.0 4.0 3.4 3.1 | - - - - - - - - - |
| 3           | 0.3                    | 5.0 4.8 4.8 | 1.4 1.4 1.1 1.5 1.4 1.6 | - - - - - - - - - |
| 4           | 0.1                    | 35 28 31   | 7.4 10 9.4 9.0 8.6 13 | - - - - - - - - - |
| 5           | 1.4                    | 19 26 26   | 7.6 6.1 6.0 3.5 3.9 6.0 | 5.2 4.1 3.0 4.7 4.7 4.8 4.7 |
| 6           | 1.8                    | 12 10 13   | 4.4 6.2 8.2 6.6 6.2 4.8 | 6.2 9.0 6.6 7.2 5.4 3.7 5.4 |
| 7           | 0.1                    | 11 10 16   | 4.4 2.6 3.1 2.6 2.8 2.4 | 4.3 3.4 7.6 5.4 4.4 4.0 3.9 |
| 8           | 1.5                    | 28 33 27   | 7.7 6.6 5.0 5.4 6.7 8.2 | 11 8.2 11 6.3 10 10 13 |
| 9           | 0.2                    | 65 55 47   | 16 16 17 16 18 17 | 22 16 22 18 15 17 15 |

Patients No. 1–9
Mean: 0.7 ± 0.2

Patients No. 5–9
Mean: 1.0 ± 0.4

Serum GH levels during iv and sc infusion (means ± SEM). The open bars below the abscissa indicate the iv and sc infusion periods. Open circles (○—○) represent all nine subjects, filled circles (●—●) represent the five subjects who received the extended sc infusion.
The difference between the iv and both the sc levels was significant ($P < 0.05$), whereas no significant difference was found between the 2 sc levels ($P > 0.15$). The results are depicted in Fig. 1. GH in our infusion system was found to be stable in the observation period. We conducted the stability study with and without the addition to the GH solution of human albumin, which had no impact on the GH stability.

Discussion

This study is the first to demonstrate significantly reduced levels of serum GH after long-term sc infusion of GH when compared with steady state serum GH levels after iv infusion.

The fact that the serum GH levels did not increase significantly during a 24-h extension of the sc infusion period seems to imply that a true steady state was reached. A very slow local accumulation and release cannot completely be ruled out, but is unlikely when noting the striking difference between sc and iv levels. Another factor could be that passage through the sc route induced some changes in the configuration of GH leading to changes in immunoreactivity. However, it has been shown that serum GH after sc injection has unaltered elution pattern following gel chromatography (Christiansen et al. 1983).

In theory, the observed difference could at least partly be due to a slower metabolic clearance rate at higher serum GH levels; however, it has been demonstrated that the elimination of GH follows first order kinetics even at high GH levels (Lauritzen et al. 1987). Finally, our stability study showed no signs of in vitro degradation of GH in the observation period.

Although this evidence is basically circumstantial we conclude that sc injected GH is degraded locally to a substantial extent. This is in contrast to insulin, of which only a small fraction is degraded if any. In addition insulin is absorbed much faster from the sc tissue than GH (Binder 1969; Lauritzen 1985). One could speculate that GH, having a molecular weight of more than 3 times that of insulin, to some extent is transported by the lymphatic system thereby being more exposed to degradation. This could also explain the slower absorption of GH than of insulin.

The potential disadvantage in sc GH therapy owing to local degradation seems to be more than balanced by other merits. Several investigators have demonstrated an increased growth rate and patient compliance using daily sc injections compared with less frequent im injections employing the same total amount of GH weekly (Kastrup et al. 1983; Albertsson-Wikland et al. 1986; Hermanussen et al. 1985). The improved growth rate could be due to the increased frequency of administration. On the other hand, the more sustained elevation in serum GH following sc injection may be an additional benefit (Christiansen et al. 1983; Jørgensen et al. 1987).

In our opinion, the present data should not be held as evidence against sc injections of GH. But one could speculate that some of the children who do not respond satisfactorily to GH therapy might be ‘GH resistant’ owing to a massive local degradation. Perhaps measurement of serum GH after GH injections should be performed in such cases.

In all instances, the recognition of sc degradation of GH should be born in mind by future investigators of the pharmacokinetic and biological properties of GH therapy.

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References


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