The metabolic clearance rate, serum half-time and apparent distribution space of authentic biosynthetic human growth hormone in growth hormone-deficient patients

J. O. L. Jørgensen, A. Flyvbjerg and J. S. Christiansen

Second University Clinic of Internal Medicine, and Institute of Experimental Clinical Research, Aarhus Kommunehospital, Aarhus, Denmark

Abstract. We studied the metabolic clearance rate (MCR) serum half-time ($t_{1/2}$) and apparent distribution space (DS) of unlabelled, authentic, biosynthetic human growth hormone (B-hGH) in 9 GH-deficient patients by means of the constant iv infusion to equilibrium technique. B-hGH was infused for 3 h at a rate of $33 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ after which the disappearance from serum of GH was followed for 1 h. The mean ± SEM values for MCR, $t_{1/2}$ and DS were: $2.3 ± 0.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $21.1 ± 1.7 \text{ min}$ and $67.6 ± 14.6 \text{ ml/kg}$, respectively. The disappearance of GH was monoeponential for the first 30 min, during which 75% of the GH had been cleared. The disappearance rate during the last 30 min of the observation period was somewhat lower, still approximately 90% of the GH had been eliminated after 60 min.

Studies on the pharmacokinetics of GH are relatively few, and may be incorrect as they have all used pituitary derived GH (P-hGH), and in most instances radiolabelled preparations. Furthermore they have mainly dealt with non-GH-deficient subjects.

Human GH is now produced by means of recombinant DNA technology, so-called biosynthetic human GH (B-hGH) (Goeddel et al. 1979). In a recent study we compared the serum profiles and short-term metabolic effects of P-hGH and B-hGH and reported no significant differences (Jørgensen et al. 1987).

In light of the possible future expansion in the clinical use of B-hGH we found it of relevance to study the metabolic clearance rate (MCR), serum half-time ($t_{1/2}$), and apparent distribution space (DS) of unlabelled monomeric B-hGH of the 22 K variant in GH-deficient patients by means of the constant intravenous infusion to equilibrium technique.

Patients and Methods

Patients

Nine GH-deficient patients were studied (Table 1). The patients had initially, before diagnosis, undergone at least one GH stimulation test: arginine infusion and/or elevation of body temperature (Christensen et al. 1984), resulting in maximum serum GH response of less than 5 µg/l. The mean ± SEM serum somatomedin-C (Sm-C) in the 9 patients prior to the study was $178 ± 25 \mu\text{g/l}$. The mean ± SEM value in normal adults with this assay is $230 ± 15 \mu\text{g/l}$ (Jørgensen et al. 1988). Four of the patients received GH replacement therapy, which was discontinued 36 h prior to the study, whereas all other medications listed in Table 1 were maintained.

Experimental design

The studies were performed in the morning with the patients in the supine position. An iv catheter was placed in each arm, one for blood sampling, the other for GH infusion. With 20-min intervals, 3 blood samples for basal serum GH measurements were drawn. A constant 3-h iv infusion of GH was then initiated, during which blood
was sampled every 15 min. After discontinuation of the infusion, blood was sampled for 1 h: every 5 min for the first 30 min, then every 15 min.

**GH preparation and analysis**

Authentic, so-called 'methionine free', biosynthetic human GH (Norditropin®; Nordisk Gentofte A/S, Gentofte, Denmark) of one single batch number was used in all the patients. This preparation consists solely of the 22K form of the GH molecule with more than 98% of it being monomeric. (Christensen et al. 1986). Two 12-lU vials (specific activity = 3 IU/mg) were dissolved in 600 ml of isotonic NaCl. The infusion rate was 33 ng · kg⁻¹ · min⁻¹ except in one patient (No. 8) who received 56 ng · kg⁻¹ · min⁻¹. The same iv infusion set and pump, with a precision of ± 1.0% (Terufusion STC-503, Rødovre, Denmark), was used in all the experiments. Serum GH was measured by RIA as previously described (Ørskov et al. 1968). This wick chromatographic RIA includes (by omission of GH-antibody) determination of incubation damage and endogenous GH-antibodies in serum (Ørskov & Seyer-Hansen 1974). None of the patients had endogenous antibodies against GH in the serum. The assay detection limit is 0.1 µg/l. Intra- and inter-assay coefficients of variation are 5.2 and 8.7%, respectively.

**Pharmacokinetic calculations**

The metabolic clearance rate (MCR) of B-hGH was calculated according to the general formula of Tait (1963):

\[ \text{MCR} = \frac{\text{infusion rate of B-hGH}}{\text{steady state serum GH}} \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}. \]

The steady state values of serum GH was calculated as the mean of the final 3 GH values during the infusion. The serum half-time \((t_{1/2})\) was estimated by means of linear regression analysis. The mean of the last 3 GH values during infusion and the GH values for all 60 min after infusion stop were included for the estimation of \(t_{1/2}\). 2. The apparent distribution space was calculated from the formula (Zilversmit 1960):

\[ \text{DS} = \frac{\text{MCR}}{0.693} \text{ ml/kg} \]

All results are expressed as mean ± SEM.

**Control of GH stability**

The stability or recovery of the dissolved GH was tested by measuring the GH concentrations contained in the infusion system at room temperature for 24 h during which samples were automatically collected at 30-min intervals.
Table 2.
Individual serum GH values before, during and after GH infusion. The basal values represent the mean of 3 samples drawn prior to GH infusion. The steady state value is the mean of the last 3 samples at the end of the 3 h infusion.

<table>
<thead>
<tr>
<th>Patient (No.)</th>
<th>Basal</th>
<th>Steady state</th>
<th>After GH infusion stop (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>24.3</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>14.3</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>4.9</td>
<td>3.9</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>29.3</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>1.4</td>
<td>23.7</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>1.8</td>
<td>11.7</td>
<td>8.0</td>
</tr>
<tr>
<td>7</td>
<td>0.2</td>
<td>12.3</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>0.2</td>
<td>55.7</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>0.1</td>
<td>31.3</td>
<td>26</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>0.7</td>
<td>23.1</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>5.0</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Results
The individual serum GH values during the study are depicted in Table 2. The 9 subjects had a mean baseline serum GH level of 0.7 ± 0.2 µg/l. Steady state was reached within 150 min after start of GH infusion in all patients with a mean value of 23.1 ± 5.0 µg/l (Table 2 and Fig. 1). The mean MCR was 2.33 ± 0.58 ml·kg⁻¹·min⁻¹ (Table 3). The calculated mean t₁/₂ was 21.1 ± 1.7 min (Table 3). The mean curve for the disappearance of GH in serum is depicted semilogarithmically in Fig. 1. The disappearance was monoxponential for the first 30 min, during which approximately 75% of the GH
had been cleared. The disappearance rate during the last 30 min, when blood was sampled every 15 min, appeared somewhat slower. At the end of the 60 min, about 90% of the infused GH was eliminated. There was no correlation between the magnitude of the steady state serum GH levels and the serum half-time in the 9 patients (r = 0.21, 2P = 0.58), and no correlation was found between either serum Sm-C and MCR (r = 0.42, 2P = 0.26) or serum Sm-C and t_{1/2} (r = 0.19, 2P = 0.63). The mean DS was 67.6 ± 14.6 ml/kg (Table 3). When comparing 5 patients with isolated GH deficiency with the remaining 4 patients no systematical differences with respect to GH pharmacokinetics were revealed.

The concentration of the dissolved GH in the infusion system was stable during the 24-h test period. The stability study was conducted with and without addition to the solution of human albumin, which had no impact on the GH stability.

### Discussion

The introduction of recombinant human GH solves the problem of limited supply of the hormone. This could mean that new, as yet unestablished, indications for GH therapy can be explored. In addition, it will now become possible to optimize GH administration to classic GH-deficient patients. Exact knowledge of the pharmacokinetics of exogenous GH is mandatory for such future studies.

We studied the pharmacokinetics of B-hGH of the authentic monomeric 22K form in GH-deficient patients. As previously mentioned, earlier studies in this field have dealt with pituitary preparations and most of these have been carried out in non-GH-deficient subjects. A number of factors might contribute to the differences found in the pharmacokinetics of P-hGH and B-hGH as well as to the differences found between results obtained in GH-deficient and non-GH-deficient subjects. Recently, Hendricks et al. (1985) elegantly demonstrated that the heterogeneous forms of P-hGH are cleared from the circulation according to the molecular size. They reported that in man the 22K form had a t_{1/2} of 19 min, a value close to our results, whereas the t_{1/2} of the dimeric form (44K) was 26.5 min and that of an even larger oligomeric form was 45 min. These results were later confirmed in an animal model (Baumann et al. 1986b). The GH preparation we used is entirely of the monomeric 22K form when analysed in vitro (Christensen et al. 1986). We have previously demonstrated that the radioimmunoassayable GH contents in serum several hours after injection of exogenous GH elute with monomeric GH on gel-filtration (Christiansen et al. 1983), and it has previously been reported that oligomerization primarily takes place in the pituitary stores (Stolar et al. 1984). In addition, substantial evidence for the presence of a specific binding protein for GH has been presented (Baumann et al. 1986a). It is at present not fully clarified to what extent GH binding proteins and receptor states influence the pharmacokinetics of exogenous GH.

Cameron et al. (1969) studied the disappearance of radioiodinated as well as unlabelled GH after both single injections and constant infusion in normal subjects. The disappearance, which was followed for 240 min, appeared to be multiexponential with both GH preparations. It was therefore concluded that a meaningful t_{1/2} could not be estimated. However, it is of interest to notice that after 60 min, within which period the disappearance curve of the unlabelled GH had been almost linear in the semilogarithmic system, more than 90% of the GH had been eliminated. At that time-point it could be assumed that a serum GH very close to the basal level (0.5–5 µg/l) had been reached, and that endogenous GH secretion influenced the re-
sults. To follow the disappearance further therefore might not be relevant to the pharmacokinetics of exogenous GH. We found the elimination of exogenous B-hGH to follow first order kinetics during the initial 30 min after cessation of GH infusion (Fig. 1). This is in agreement with the results obtained by Owens and coworkers (1973) in normal subjects.

After 30 min the disappearance rate of B-hGH, which was then followed only at 15-min intervals, appeared to be slower (Fig. 1). This might in part be due to endogenous GH immunoreactivity, and it should be noticed that this reduced disappearance rate was most pronounced in subjects No. 1, 5 and 6, who rapidly reached a low serum GH level, which then remained almost constant (Table 2).

Taylor et al. (1969) calculated the MCR of GH in normal subjects and in several patient groups (not including GH deficiency) using constant infusion of labelled GH. The MCR in normal subjects was 229 ml/min, whereas a significantly reduced MCR in insulin-dependent diabetic patients was found. The latter is interesting, since the elevated serum GH levels seen in these patients are traditionally ascribed to hypersecretion of the hormone (Gerich 1984; Ørskov 1985).

Observations in GH deficiency are relatively scarce. MacGillivray et al. (1970) Calculated the mean MCR to be 109 ml·min⁻¹·m⁻² in 13 GH-deficient subjects when employing infusion of labelled GH. When corrected for body surface area, our data yields MCR of 86.4 ml·min⁻¹·m⁻². It is in accordance with observations obtained with iodinated insulins which exhibit significantly greater serum half-lives than native insulins (Ørskov & Christensen 1969). Kowarski et al. (1971) estimated the MCR in 3 hypopituitary patients and reached values comparable to our results.

In conclusion, we have found that when administered as a constant iv infusion to GH-deficient subjects, B-hGH has a MCR of 2.3 ± 0.6 ml·kg⁻¹·min⁻¹, a t₁/₂ of 21.1 ± 1.7 min, and a DS of 67.6 ± 14.6 ml/kg. These data may be of future help in improving the ways of administering exogenous GH.

Acknowledgments

We are greatly indebted to Inga Bisgaard, Kirsten Rasmussen and Joan Hansen for their technical assistance and to Anette Andersen for secretarial help.

References


Received March 29th, 1988.
Accepted September 16th, 1988.

Dr Jens Otto Lunde Jørgensen,
Second University Clinic of Internal Medicine,
Aarhus Kommunehospital,
DK-8000 Aarhus C,
Denmark.