Collagen metabolites in the prediction of response to GH therapy in short children

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Abstract

To evaluate the role of collagen metabolites in the prediction of the response to GH treatment we measured the serum concentrations of the C-terminal propeptide of type I procollagen (PICP) and the N-terminal propeptide of type III procollagen (PIIINP) with specific RIAs in 35 short children (16 boys) before and after 5 days, 5 weeks and 3 months of GH therapy. The mean age of the children was 10.3 years (range 1.9–16.4 years) and the bone age ranged from 1.2 to 12.5 years (mean 7.6 years). The initial mean relative height (RHI) was 3.6 SDS (range 6.6 to 2.4 S.D.). Nineteen children were found to have GH deficiency (GHD; peak GH responses in two pharmacological tests <10 μg/l), while the remaining 16 were considered to have undefined short stature (USS). The children were treated with recombinant human GH (0.1 U/kg given subcutaneously at bedtime 6–7 times/week). The increases in RHI over the first 6 and 12 months of therapy were used as response measures. There was already a significant increase (P < 0.001) in both the serum PICP and PIIINP levels at 5 days, and the concentrations continued to rise up to 3 months, PICP levels rising less than the PIIINP levels. In the whole group the RHI over 6 months correlated most strongly with the absolute PICP concentrations at 3 months (r_s = 0.59; P < 0.05), while the absolute PIIINP concentrations at 3 months showed the strongest relation to the one year RHI (r_s = 0.69; P < 0.001). In the GHD group the 6 month RHI was most strongly related to the absolute PICP concentration at 3 months (r_s = 0.59; P < 0.05). In the USS group the absolute PICP concentrations at 3 months correlated most strongly with the one year RHI (r_s = 0.82; P < 0.01). Significant correlations were also observed between the absolute PIIINP levels at 3 months and the 6 month RHI (r_s = 0.60; P < 0.05) and 12 month RHI (r_s = 0.76; P < 0.01) in this group. These results show that GH therapy results in an unequivocal increase in circulating concentrations of PICP and PIIINP. The serum PICP and PIIINP concentrations may be of value in the prediction of the long-term response to GH therapy.

Introduction

The increased availability of biosynthetic human growth hormone (GH) and the possibility of using GH for treatment of a variety of growth disorders has increased the need for biochemical predictors of the response to growth-stimulating therapy. Present knowledge indicates that the GH-induced insulin-like growth factor-I (IGF-I) response may be a useful predictor of response to therapy in children with undefined short stature (USS) but not in GH-deficient (GHD) children (1, 2). A preliminary report suggests, however, that pretreatment serum IGF-I concentrations correlate inversely with growth response to GH in patients with hypopituitarism, if the IGF-I levels are measured with appropriate methodology (3). Serum concentrations of the N-terminal propeptide of type III collagen (PIIINP) have been found to be related to growth rate in healthy children (4). We and others have previously shown that the measurement of serum PIIINP, particularly in children receiving exogenous GH, may be a potential early indicator of the growth response to therapy and may provide useful additional biochemical information during growth (5–7). Serum concentrations of the C-terminal propeptide of type I collagen (PICP) have also been reported to be abnormally low in GHD children (8, 9), and there is some evidence that serum PICP could be more useful in assessing growth than serum PIIINP (8, 10). To evaluate the collagen metabolites in the prediction of the response to GH treatment, we measured the serum concentrations of PIIINP and PICP with specific RIAs in short children treated with GH.

Materials and methods

Study subjects

Thirty-five children, 16 boys and 19 girls, aged 1.9–16.4 years, were studied. All had been referred to the
Department of Pediatrics, University of Oulu, for investigation of short stature. The growth of the children had been followed from birth at child welfare clinics by a minimum of one annual measurement. Those older than 6 years were measured twice a year at school. The relative height (RHI) score of all were below $-2.4$ S.D., based on the Finnish growth charts (11). Beginning at about 12 months before the start of GH therapy, height was measured two to four times in the Endocrine Clinic using the same Harpenden stadiometer as was used during the study. The mean $\pm$ s.d. absolute growth velocity before treatment was $4.5 \pm 1.8$ cm/year and the relative growth velocity score $2.0 \pm 1.9$ based on the height velocity charts of Tanner et al. (12). A radiograph of the hand and wrist was taken and the bone age estimated according to the criteria of Greulich & Pyle (13). All had retarded bone ages ranging from 1.2 to 12.5 years. GH was defined as a serum GH response less than $10 \mu g/l$ in response to both clonidine- ($0.15 mg/m^2$ by mouth) and insulin- ($0.1 IU/kg i.v.$) induced hypoglycaemia. Nineteen children were found to have GHD, while the remaining 16 were considered to have USS. The mean $\pm$ s.d. RHI score of the children with GHD was $-3.7 \pm 1.0$, while the mean absolute growth velocity before the treatment was $4.1 \pm 2.0$ cm/year. In the group with USS the mean RHI score was $-3.6 \pm 1.1$, and the absolute growth velocity before treatment was $4.8 \pm 1.3$ cm/year (Table 1). All of the girls had the 46XX karyotype. Written informed consent was obtained from the parents of each child and the study was carried out according to the provisions of the Declaration of Helsinki.

### Study protocol

The children were treated with biosynthetic human GH given at bedtime, 0.1 U/kg subcutaneously 6 to 7 times a week. Blood samples for the measurement of serum PICP and PIIINP were collected before the commencement of treatment and at 5 days, 5 weeks and 3 months during the therapy. The interval between the last GH injection and sampling was 17–18 h. Throughout the study, height was measured to the nearest 0.1 cm with a wall-mounted Harpenden stadiometer at the same time of the day. Height velocities are expressed as annualized growth velocities. The relative height velocities are expressed in s.d. score for chronological age in the children.

### Methods

The serum concentrations of PICP were measured in duplicate $50 \mu l$ serum samples with an equilibrium RIA kit (ORION Diagnostica, Oulunsalo, Finland) (14). The reference range of serum PICP is $160–540 \mu g/l$ in girls and boys aged 1–3 years, $220–350 \mu g/l$ in those aged 3–9 years, $220–380 \mu g/l$ in girls aged 9–11 years, $220–420 \mu g/l$ in boys aged 9–13 years, $180–420 \mu g/l$ in girls aged 11–14 years, $220–500 \mu g/l$ in boys aged 13–16 years and $90–200 \mu g/l$ in girls aged 14–16 years. The sensitivity of the assay was $1.5 \mu g/l$. The intra-assay and interassay coefficients of variation were $3$ and $5\%$ respectively.

Serum concentrations of PIIINP were measured in duplicate $100 \mu l$ serum samples with an equilibrium RIA kit (ORION Diagnostica) (4). The reference values of serum PIIINP were $3.7–12.2 \mu g/l$ in girls and $3.6–11.8$ in boys aged 2–10 years, $4.9–17.2 \mu g/l$ in girls and $3.1–13.3 \mu g/l$ in boys aged 11–13 years, and $2.0–10.0 \mu g/l$ in girls and $5.8–21.5 \mu g/l$ in boys aged 14–17 years (5). The sensitivity of the assay was $0.2 \mu g/l$. Both the intra-assay and interassay coefficients of variation were less than $5\%$ at the concentrations found during the study.

Plasma IGF-I concentrations were measured using commercial kits after acid ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The sensitivity of the assay was $0.5$ nmol/l. The intra-assay coefficient of variation was $5.1\%$ and the inter-assay variation less than $10\%$. The reference range of plasma IGF-I was $3.0–50$ nmol/l in children aged 3–6 years, $5.5–70.0$ nmol/l in 6–11-year-old boys, $10.0–112.5$ nmol/l in girls aged 6–11 years, $7.0–87.5$ nmol/l in 11- to 13-year-old boys, and $24.8–170$ nmol/l in girls of the same age, whereas the range in older children was $22.5–147.5$ nmol/l.

### Statistical methods

The significance of the difference between two groups was estimated by the $t$-test and the correlation between two parameters by the non-parametric Spearman’s correlation analysis. $P$ values less than 0.05 were considered statistically significant.

### Results

Serum PICP concentrations at different times after the initiation of GH therapy are shown in Fig. 1. The mean

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Bone age (years)</th>
<th>RHI (SDS)</th>
<th>Absolute growth velocity before treatment (cm/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHD ($n = 19$, 10 boys)</td>
<td>$10.7 \pm 4.4$</td>
<td>$7.9 \pm 3.8$</td>
<td>$-3.7 \pm 1.0$</td>
<td>$4.1 \pm 2.0$</td>
</tr>
<tr>
<td>USS ($n = 16$, 9 boys)</td>
<td>$10.2 \pm 3.1$</td>
<td>$7.6 \pm 3.0$</td>
<td>$-3.6 \pm 1.1$</td>
<td>$4.8 \pm 1.3$</td>
</tr>
</tbody>
</table>
pretreatment concentration of serum PICP was 271.6 ± 121.0 µg/l in the GHD children and 313.8 ± 124.3 µg/l in those with USS. There was already a significant increase (P < 0.001) in serum PICP levels at 5 days in both groups (356.6 ± 148.6 µg/l in GHD and 348.9 ± 143.4 µg/l in USS), and the concentrations continued to rise up to 3 months (460.9 ± 147.8 µg/l and 453.5 ± 110.9 µg/l respectively). The increases in RHI over the first 6 and 12 months of therapy were used as response measures. There was a significant correlation between the change in RHI over 6 months and serum PICP concentrations at 3 months (Fig. 2).

The 6 month RHI was most strongly related to absolute PICP concentration at 3 months (r_s = 0.59; P < 0.05) in the children with GHD. In the USS group the absolute PICP concentrations at 3 months correlated most strongly with the one year RHI (r_s = 0.82; P < 0.01).

Serum PIIINP concentrations at different times after the initiation of GH therapy are shown in Fig. 3. The mean pretreatment serum PIIINP concentrations were 7.3 ± 2.9 µg/l in the GHD children and 7.4 ± 3.2 µg/l in the group with USS. Also serum PIIINP levels had already increased significantly (P < 0.001) at 5 days in both groups (10.6 ± 5.4 µg/l in GHD and 9.4 ± 3.9 µg/l in USS), and continued to rise up to 3 months (13.4 ± 5.8 µg/l and 11.8 ± 4.2 µg/l respectively). The increase tended to be greater in children with GHD than those with USS. Figure 4 shows the correlation between the change in RHI over 12 months and serum PIIINP concentrations at 3 months. In the USS group a significant correlation was observed between the absolute PIIINP levels at 3 months and 6 month (r_s = 0.60; P < 0.05) and 12 month RHI (r_s = 0.76; P < 0.01).

The pretreatment concentrations of plasma IGF-I ranged from 6.5 to 60 nmol/l in the GHD children and from 9.8 to 72.5 nmol/l in the children with USS. All but two GHD children had low IGF-I levels. No significant correlation was found between RHI over 3, 6 or 12 months and the concentrations of IGF-I (data not shown).
Discussion

Type I collagen is the most abundant of the interstitial collagens found in mineralized bone and soft tissues, whereas type III collagen mostly occurs only in soft connective tissue (15, 16). Both serum PICP and PIIINP concentrations have been found to reflect growth and correlate with height velocity in prepubertal children (6, 11). We and others have also shown that serum PIIINP levels can predict the response to GH therapy (5–7).

Our present results show that the serum concentrations of both PICP and PIIINP had already increased significantly after 5 days of GH therapy and the concentrations continued to rise up to 3 months. This was seen in cases of both GHD and USS. Similar increases in PICP concentrations have previously been reported by Trivedi et al. (10) and Carey et al. (8), but their study populations comprised 14–15 children with GHD, no children with USS were included, only PICP concentrations were observed and no comparison with PIIINP levels was made. We could not separate GHD children from those with USS on the basis of decreased basal PICP levels as Carey et al. reported (8), although the GHD children tended to have lower basal levels. Thus it is not possible to use serum PICP levels in the differential diagnosis of GHD. This is not surprising, since the PICP reflects mainly bone metabolism. The markers show acceleration of growth, but bone collagen formation is not directly regulated by GH.

We have previously reported a clear correlation between the GH-induced increase in serum PIIINP levels at 5 weeks and growth velocity after 6 and 12 months of GH therapy (5). Now we observed a close correlation between the change in RHI over 12 months and the absolute PICP concentrations at 3 months. The absolute PIIINP concentrations at 3 months showed, however, the strongest relation to the 12 month increase in RHI. Our results indicate that measurement of serum PIIINP concentrations may provide a potential early indicator of growth response to therapy. Our findings that GH therapy results in an unequivocal increase in circulating concentrations of PICP and PIIINP suggest that both serum PICP and PIIINP concentrations may be of...
value in the prediction of the long-term response to GH therapy.

Do we need biochemical early indicators of growth response to therapy? Although children with GHD have been treated with replacement therapy for over 30 years and children with USS for about 10 years, major predictors of the response to treatment have not been clearly defined. To date, determinations of body height and calculation of growth rates are still the major parameters for the assessment of the response to GH therapy. However, at least 6 months of therapy, preferably 12 months, is needed to evaluate reliably whether exogenous GH has a meaningful growth-promoting effect in a slowly growing child. In cases of USS it is known that some children will benefit from GH therapy and some will not. One year on GH therapy is expensive and tedious. In this group of patients in particular, biochemical indicators of the growth response to therapy could be useful. One month of therapy may be too short a time to draw conclusions, but in 3 months it is possible to combine clinical and biochemical data for the decision making. Serum concentrations of both PIIINP and PICP may be of use for this purpose.

It is well known that GH secretion increases in puberty and it has been suggested that the dose of GH should be individually adjusted in each patient. Jensen et al. (17) have found dose-dependence for PIIINP, but dose-dependence was not so obvious for PICP. In our study, concentrations of serum PICP increased less than PIIINP levels, suggesting that PIIINP may be a better predictor than PICP. However, both may be useful for optimization of the GH dose in individual patients. We have recently developed an assay for the intact N-terminal propeptide of human type I procollagen (18). Unfortunately there were no samples left to study whether this assay would be more sensitive than that for PICP in these patients. Our concomitant measurements of serum PICP and PIIINP in GHD patients during GH therapy, although we did not particularly study dose-dependency, suggest that it may be possible by these measurements to identify GH-responsive short children and adjust their dose of exogenous GH. Future research will provide more information on whether these candidate proteins can be used, along with the auxiological measurements, for predicting the long-term response to GH therapy.

References

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