Preliminary Report

The effect of recombinant human growth hormone treatment on bone and mineral metabolism in haemodialysis patients

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Abstract

Background. Uraemia and chronic haemodialysis are associated with an abnormal growth hormone (GH)–insulin-like growth factor (IGF) axis which may contribute to malnutrition and renal bone disease. Short-term studies have shown a beneficial effect of treatment with recombinant human growth hormone (rhGH) on nutritional status in patients on haemodialysis. In the present study, we evaluated the effect of rhGH on bone and mineral metabolism.

Methods. Twenty chronic malnourished patients on haemodialysis took part in a double-blind, placebo controlled trial with subcutaneous injections of rhGH (4 IU/m²/day) or placebo for 6 months.

Results. During rhGH treatment, serum IGF-1 increased 264 ± 52% (mean ± SEM) (P < 0.008). There were no significant changes in biochemical markers of mineral metabolism (serum ionized calcium, phosphate and parathyroid hormone). Among markers of bone metabolism, there was a significant increase in serum procollagen type I C-terminal propeptide (maximum 155 ± 8%, P < 0.001) and no significant changes in serum alkaline phosphatase. Bone densitometry showed a significant decrease in whole body bone mineral content (95.7 ± 1.2%) after 6 months treatment. The effects on the proximal femur were not significant.

Conclusion. The effects of 6 months treatment with rhGH seen in this study are best explained by a GH- or IGF-1-induced increased bone turnover. Long-term treatment in larger cohorts followed by bone densitometry and, preferentially, bone histomorphometry are needed to evaluate whether this is a beneficial effect in haemodialysis patients.

Key words: alkaline phosphatase; bone mineral density; bone remodelling; C-terminal type I collagen propeptide; growth hormone treatment; haemodialysis

Introduction

Recombinant human growth hormone (rhGH) has proven an effective treatment of growth hormone (GH) deficiency in adults [1] whereby low lean body mass and bone mass, as well as reduced muscular strength and exercise capacity, can be reversed. Some of the symptoms associated with uraemia and chronic haemodialysis (HD) resemble those of GH deficiency, and short-term rhGH treatment has therefore been used in uraemic animal models, children and adults with renal failure [2–6] with promising effects.

Patients with renal failure have normal or even elevated serum levels of GH and insulin-like growth factor (IGF), but the bioactivity of IGF is reduced because of an excess of IGF-binding proteins and diminished responsiveness to GH and IGF [2].

GH has a profound effect on bone metabolism in normals throughout life. In vitro, rhGH increases differentiation and proliferation of osteoblasts [7], and in vivo rhGH treatment increases bone turnover evaluated by biochemical markers [8]. Uraemia and HD are associated with multiple pathological mechanisms affecting bone and mineral metabolism, but decreased 1,25-dihydroxyvitamin-D₃ [1,25(OH)₂D₃] synthesis and phosphate retention are the most prominent features and are associated with low bone density [9]. The effect of rhGH treatment on bone and mineral metabolism in adult patients with renal failure is not known, but potentially it may have an indirect beneficial effect by enhancing muscle mass and a direct effect by activating bone mineralization and bone remodelling, although the latter also may be deleterious in high turnover renal bone disease.

Based on the promising effect of short-term rhGH treatment of uraemic subjects [2–6], we performed a study to evaluate the effect of 6 months treatment with rhGH in patients on chronic HD. To test the hypothesis of a GH-stimulating effect on bone remodelling, we measured bone density and biochemical markers of bone and mineral metabolism before and after treatment.
Subjects and methods

Patients

Thirty-one patients on chronic HD for at least 6 months and with a dialysis efficiency >1.0 measured by KT/V were included in the study at four dialysis centres. Participants were all clinically malnourished and were of reduced weight compared with the pre-dialysis situation. Patients with diabetes mellitus, tertiary hyperparathyroidism, malignancy, active autoimmune disease or those treated with immunosuppressive drugs in the preceding 6 months were excluded. The study was approved by the local ethics committee (journal no. 92/33 MC). The investigation was conducted in accordance with the Declaration of Helsinki II and the guidelines of Good Clinical Practice.

Study design

The study was a double-blind, randomized, placebo-controlled, multi-centre study, with a duration of 6 months. Randomization was done in blocks of four.

rhGH (Norditropin®; Novo Nordisk A/S, Gentofte, Denmark) was administered as daily subcutaneous (S.C.) injections at 8 p.m. at a dosage of 1.0 IU/m²/day. Placebo preparations were administered in a similar fashion and consisted of volume of solvent identical to that of the rhGH preparation. Surface area was calculated as $\sqrt{\text{weight} \times \text{height}^2}$. Serum intact parathyroid hormone (1–84) (PTH) was measured by in vitro method.

Study protocol

Patients were included during a 2-year period. Physical examinations were performed monthly in the HD department. Blood samples were collected after an overnight fast and immediately before HD. In order to reduce analytical variation, samples from each patient were analysed in the same run. Safety parameters were measured monthly, and other biochemical parameters every second month. In all patients, dual-energy X-ray absorptiometry (DXA scans) were performed at one centre on a non-dialysis weekday at baseline and after 6 months.

Every participant was kept on their normal medication and alterations were allowed according to normal clinical decision. Changes in hydroxylated vitamin D [1α(OH)2D3] and phosphate binder treatment (CaCO₃ and aluminium aminocacetate) were only allowed in cases where the serum ionized calcium or phosphate concentration exceeded the therapeutic range (S-Ca²⁺ 1.20–1.35 mmol/l, S-phosphate >2.0 mmol/l).

Methods

DXA scans to assess bone mineral content [BMC (g)] and density [BMD (g/cm²)] were performed at different anatomical regions [whole body and hip (total and femoral neck) using a Hologic QDR 2000/W scanner. All scans were performed in single beam mode. The in vivo precision (CV) of BMD measurements of whole body and femur was 1.6 and 0.6% respectively.

Serum IGF-I was measured after extraction with HCl/ethanol in an immunofluorometric sandwich assay with two monoclonal antibodies using the Delphia principle and an AutoDelphia reader (Wallac, Turku, Finland). The detection limit was 2.5 ng/l. Intra- and interassay CV were <5 and <10% respectively.

Serum total alkaline phosphatase activity (AP) was measured spectrophotometrically using p-nitrophenylphosphate as substrate according to the method recommended by the Scandinavian Committee on Enzymes. The intra- and interassay CV were 2.5 and 5% respectively.

Serum bone isoenzyme alkaline phosphatase (bone AP) was measured spectrophotometrically after lectin precipitation (Boehringer Mannheim, Germany). The intra- and interassay CV were 9.8 and 10.0% respectively.

Serum procollagen type I C-terminal propeptide (PICP) was measured by radioimmunoassay (RIA) (Farmos Diagnostica, Oulu, Finland). The intra- and interassay CV were 3.2 and 7.0% respectively.

Serum intact parathyroid hormone (1–84) (PTH) was measured by RIA (Farmos Diagnostica, Oulu, Finland). The intra- and interassay CV were 9 and 10% respectively.

Serum ionized calcium and phosphate and safety parameters (blood haemoglobin, plasma sodium, potassium, creatinine, alanine aminotransferase) were analysed according to standard laboratory methods.

Statistical methods

The normal distribution of data was checked using the Kolmogorov-Smirnov goodness of fit test. When indicated, logarithmic transformation of the data was used (serum AP and bone AP). All results are presented as mean ± SEM and mean differences as percentage of baseline ± SEM. Baseline data were compared using the unpaired two-tailed t-test. Variations in biochemical markers due to differences between groups and treatment response over time were identified by repeated measures analysis of variance (ANOVA). A post-hoc analysis was done using the paired two-tailed t-test against day 0 within each group. Changes in DXA measurements were evaluated by the paired two-tailed t-test. All calculations were done using SPSS (Statistical Package for Social Sciences).

Results

Twenty of the 31 patients, 12 men and eight women, aged 46 (range 18–63) years, completed the study (Table 1). Eleven patients dropped out because of kidney transplantation (three patients in the rhGH group and two in the control group), adverse events (claudication intermittent (one patient in the placebo group), arthritis/arthrosis (one patient in the rhGH group) and non-compliance (two patients in the rhGH group)]. Two patients were excluded before treatment was initiated because of severe hepatic calcification demonstrated at the first DXA scan (one patient) and myocardial infarction (one patient). Data from these 11 patients were excluded from the analysis.

There were no significant differences in age, sex and duration of renal failure between treatment groups.

Baseline parameters are presented in Table 1.
Growth hormone treatment in haemodialysis patients

Table 1. Baseline patient characteristics, biochemistry and osteodensitometry (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Growth hormone</th>
<th>Placebo</th>
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<tbody>
<tr>
<td></td>
<td><em>n=9</em></td>
<td><em>n=11</em></td>
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<tr>
<td>Patients</td>
<td></td>
<td></td>
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<tr>
<td>Male/female</td>
<td>5/4</td>
<td>7/4</td>
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<tr>
<td>Age (range) (years)</td>
<td>38 (18–63)</td>
<td>48 (18–68)</td>
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<tr>
<td>Haemodialysis duration (months)</td>
<td>50 (11–140)</td>
<td>72 (10–293)</td>
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<tr>
<td>Diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>End-stage renal disease</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Others</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Treatment influencing mineral metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminium acetate</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Hydroxylated vitamin D</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Biochemistry</td>
<td></td>
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</tr>
<tr>
<td>S-IGF-1 (ng/ml)</td>
<td>200 ± 20</td>
<td>286 ± 37</td>
</tr>
<tr>
<td>S-Ca&lt;sup&gt;2+&lt;/sup&gt; (mmol/l)</td>
<td>1.17 ± 0.09</td>
<td>1.24 ± 0.04</td>
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<tr>
<td>S-P (mmol/l)</td>
<td>1.75 ± 0.15</td>
<td>2.01 ± 0.17</td>
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<tr>
<td>S-PTH (pmol/l)</td>
<td>10.0 ± 3.5</td>
<td>8.7 ± 2.6</td>
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<tr>
<td>S-PICP (ng/ml)</td>
<td>246 ± 51</td>
<td>232 ± 32</td>
</tr>
<tr>
<td>S-AP (U/l)</td>
<td>338 ± 110</td>
<td>175 ± 20</td>
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<tr>
<td>S-bone AP (U/l)</td>
<td>212 ± 107</td>
<td>48 ± 7</td>
</tr>
<tr>
<td>S-PIIINP (µg/l)</td>
<td>7.8 ± 1.3</td>
<td>9.0 ± 1.5</td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
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<tr>
<td>Height (cm)</td>
<td>168 ± 3</td>
<td>166 ± 3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.9 ± 3.7</td>
<td>60.4 ± 2.9</td>
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<tr>
<td>Bone densitometry</td>
<td></td>
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<tr>
<td>Whole body</td>
<td></td>
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<tr>
<td>BMC (g)</td>
<td>1746 ± 138</td>
<td>2016 ± 148</td>
</tr>
<tr>
<td>Area (cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1881 ± 101</td>
<td>2037 ± 95</td>
</tr>
<tr>
<td>BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.918 ± 0.029</td>
<td>0.988 ± 0.031</td>
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<tr>
<td>Femur neck</td>
<td></td>
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<tr>
<td>Z-score</td>
<td>−1.77 ± 0.27</td>
<td>−1.51 ± 0.32</td>
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<tr>
<td>BMC (g)</td>
<td>3.26 ± 0.25</td>
<td>3.77 ± 0.36</td>
</tr>
<tr>
<td>Area (cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>5.21 ± 0.23</td>
<td>5.44 ± 0.26</td>
</tr>
<tr>
<td>BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.626 ± 0.042</td>
<td>0.686 ± 0.042</td>
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</table>

No significant differences were observed between groups in baseline parameters.

Medication

There were no significant changes from start to month 6, although the mean dosage of 1α(OH)D<sub>3</sub> was reduced by 45% after the first 3 months and remained low in the rhGH-treated group (2.86 µg/week at start and 1.56 µg/week at month 6 (*P* < 0.12)). The changes in CaCO<sub>3</sub> and aluminium aminoacetate treatment were much less marked in both groups.

Anthropometry

There were no changes in weight and height in any of the groups.

Biochemistry

Serum IGF-1 increased significantly in the rhGH-treated group (maximum 304 ± 43%, *P* < 0.008) and declined to 77 ± 8% (*P* < 0.02) (ANOVA *P* < 0.001) in the control group, but was still within the normal range.

There were no significant changes in serum ionized calcium, phosphate and PTH (Figure 1). There was a tendency towards an increase in ionized calcium [maximum at month 3 (114 ± 5%)] and in phosphate [maximum at month 4 (126 ± 14%)] in the rhGH-treated group.

Bone formation evaluated by the biochemical

![Fig. 1. Effect of treatment with either growth hormone (---) or placebo (---) for 6 months on serum PTH, serum ionized calcium and serum phosphate in patients on chronic haemodialysis. Results are expressed as percentage change from baseline (mean ± SEM). Baseline values are given in Table 1.](image-url)
markers serum PICP, serum AP and serum-bone AP is shown in Figure 2. Serum PICP increased significantly (maximum 155 ± 8%, \( P < 0.001 \)) (ANOVA \( P < 0.001 \)). There was a decrease in serum AP and bone AP in the rhGH-treated group, followed by a secondary increase which did not reach significance.

Serum PIIINP, a marker of extraskeletal collagen synthesis, increased significantly in the rhGH-treated group (maximum 221 ± 21%, \( P < 0.001 \)) (ANOVA \( P < 0.001 \)) (Figure 2).

Bone densitometry

Compared with age- and sex-matched healthy controls (Z-scores), both groups were characterized by a low BMD in the femur (Table 1). There was a significant decrease in whole body BMC (95.7 ± 1.2%, \( P < 0.05 \)) in the rhGH-treated group. Because of a concomitant decrease in whole body bone area (96.8 ± 1.2%, \( P < 0.05 \)), the decrease in whole body BMD was not significant (98.9 ± 0.5%, \( P < 0.13 \)) (Figure 3). The effects on the femur neck and total femur were not significant.

Discussion

The dose of rhGH (4 IU/m²/day) used in the study is higher than recommended for GH-deficient adults, but was chosen because of the insensitivity of target organs to GH reported in chronic renal failure. It has been demonstrated that rhGH treatment of adult patients with chronic renal failure on long-term HD is associated with changes in bone metabolism.

However, we did not find significant increases in serum calcium and phosphate as expected from studies on rhGH treatment of GH-deficient patients [10] and normals [11]. When normal renal function is present, GH indirectly increases the renal re-absorption of phosphate through IGF-1 [12], whereas no effect of GH on renal calcium re-absorption has been reported.

Based on elevated biochemical bone markers and decreased BMC in GH-deficient adults after 12 months rhGH treatment, it has been proposed that GH stimulates bone turnover, whereby serum calcium and renal calcium excretion increase due to mobilization of skeletal calcium [13]. In HD patients, short-term GH treatment has been followed by unaltered serum calcium and a decline in serum phosphate and PTH [3,4]. We observed a non-significant increase in serum calcium and in phosphate, which was maximal after 3 months. As discussed below, high bone turnover may have been present in our study, and the lack of a significant increase in serum calcium can be a statistical type 2 error or caused by the concomitant reduction in vitamin D treatment in the rhGH-treated group which may have blunted the effect.

In recent years, biochemical markers reflecting bone metabolism have been studied extensively as under ideal circumstances, they provide a dynamic and non-invasive method to study changes in bone turnover. The markers, serum PICP, AP, bone AP and osteocalcin have been validated against bone histomorphometry in a variety of metabolic bone diseases, and correlate well with bone formation. In patients with renal failure, AP, osteocalcin and PICP have proven to be effective markers [14–16], although the latter may not be useful in cases of severe aluminium overload or severe secondary hyperparathyroidism [15,16].

In the present study, there were a marked increase...
in serum PICP and a non-significant increase in AP and bone AP. PICP is derived from collagen I synthesis and is cleared from the circulation by liver endothelial cells via mannose receptors. Serum levels of PICP are believed to reflect mostly synthesis in bone, and the contribution from soft connective tissue is small [17]. AP and the bone isoenzyme (bone AP) are produced by osteoblasts, but also by other cell types. The function of AP is not known with certainty, but it is considered to be important in the mineralization process. In contrast to PICP, the routes of AP elimination are not known, especially in HD patients. This may be the reason for the observed difference between PICP and AP, although differences between markers have also been observed in situations where bone remodelling is acutely affected, such as in normals treated with 1,25(OH)2 D3 [18] or GH [8]. Long-term rhGH treatment of GH-deficient patients has also produced differences, with an increase in PICP preceding an increase in AP [13].

Non-bone collagen synthesis is also stimulated by rhGH, as reflected by the 2-fold increase in serum PIIINP, which is in accordance with other studies on GH treatment [19]. Although mainly derived from bone, PICP is not specific for bone collagen synthesis [17], and an increase in soft tissue collagen synthesis in the present study cannot be excluded as a contributor to the observed rise in PICP. There is a lack of good biochemical markers of bone resorption in HD patients, but measurement of collagen catabolism products in serum as pyridinolines or cross-linked telopeptides of type I collagen (ICTP and INTP) may have enhanced the information on bone remodelling in the present study.

Uraemia and HD are associated with a high frequency of renal osteodystrophy, and osteodensitometry has shown decreased BMD and BMC in patients compared with controls [9]. Our patients were on an average 1.6 SD below age- and sex-matched controls. rhGH treatment decreased the whole body BMC significantly and the whole body BMD non-significantly, but had no effect on the femoral region. The effect on whole body BMC most probably reflects loss of bone mineral, as changes in bone water caused by fluid retention or small changes in bone composition caused by rhGH treatment are not likely to affect the result of osteodensitometry [20]. Our results are in accordance with those of studies on rhGH-treated GH-deficient patients [13,19,21]. The effect of GH in the present study, as well the other studies mentioned, is best explained as a result of increased bone turnover. Bone remodelling, the process whereby old bone is renewed, is essential for maintenance of the biomechanical properties of the skeleton. An increase in bone turnover caused by activation of new remodelling cycles is characterized by a reversible increase in the remodelling space and, thereby, a reversible decrease in bone mass. The long-term effects depend on the existence of a positive or negative bone balance per remodelling cycle. Confirmation of such effects will require bone biopsies for histomorphometry or longer treatment time with repeated osteodensitometry.

Data from the present study do not allow the conclusion that GH has a beneficial effect on bone in HD patients. The time course of biochemical bone and mineral markers and osteodensitometry, however, fit well with studies on rhGH treatment of GH-deficient patients where longer treatment time resulted in gain of bone mineral [19,22]. Renal osteodystrophy is very heterogeneous, and is characterized by high as well as by low bone turnover disease. A GH-induced increase in bone remodelling activation frequency may enhance the risk of trabecular perforations and increased bone loss if there is negative bone balance per remodelling cycle. Further studies employing long-term treatment in larger cohorts together with bone histomorphometry are needed to establish the effect, especially in subgroups of patients with renal osteodystrophy.

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