Effects of IGF-I combined with GH on glucocorticoid-induced changes of bone and connective tissue turnover in man

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Abstract

Chronic glucocorticoid therapy results in negative bone and connective tissue balance. To assess the effects of GH and a combination of IGF-I and GH, 24 healthy male volunteers received in a double blind fashion either recombinant human GH (0·3 IU/kg per day s.c.), or a combination of GH (0·3 IU/kg per day s.c.) and IGF-I (80 μg/kg per day s.c.) or placebo (saline s.c.) during 6 days of methylprednisolone (0·5 mg/kg per day) treatment. Methylprednisolone decreased serum osteocalcin concentrations during placebo treatment from 32·9 ± 2·1 to 9·0 ± 1·4 µg/l (P<0·0001), indicating diminished osteoblast activity, and procollagen type I (PICP) and procollagen type III (PIIINP) to 46 and 70% of baseline respectively (P<0·005), indicating diminished bone (PICP) and soft tissue collagen synthesis (PIIINP). Urinary excretion of pyridinoline, deoxypyridinoline and hydroxyproline increased during treatment with methylprednisolone alone, indicating increased bone resorption (P<0·05 or less). The combination of GH and IGF-I resulted in a significant blunting of the methylprednisolone effect on serum PICP and PIIINP concentrations (P<0·005 or less vs placebo); this effect was in part due to IGF-I, since serum PICP concentrations decreased less in the combination group than during GH treatment alone (P<0·05). In the groups receiving GH and GH combined with IGF-I, urinary hydroxyproline excretion increased more when compared with methylprednisolone alone (P<0·05 or less).

These findings demonstrate that only the combination of GH and IGF-I, but not GH alone, markedly counteracts diminished bone and body collagen synthesis caused by glucocorticoids, whereas connective tissue resorption is enhanced during treatment with GH alone and in combination with IGF-I.

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Introduction

Glucocorticoids in patients with Cushing’s syndrome or during therapeutic administration exert side-effects such as osteopenia (Libanati & Baylink 1992). Bone loss is directly related to impairment of bone formation and to increased bone resorption (Reid 1989, Lukert & Raiz 1990, Libanati & Baylink 1992). Anabolic hormones such as growth hormone (GH) and insulin-like growth factor-I (IGF-I) may counteract glucocorticoid-induced bone and connective tissue turnover (Johansson et al. 1996). Both GH (Amato et al. 1993, Beshyah et al. 1994, Johansson et al. 1993) and IGF-I (Ebeling et al. 1993) have been demonstrated to increase both bone formation and bone resorption. Treatment of human osteoblast-like cells with cortisol decreased IGF-I mRNA levels (Swolin et al. 1996). Dexamethasone impaired GH-induced stimulation of local secretion and paracrine action of IGF-I and reduced the increase in IGF receptor and GH receptor expression in rat chondrocytes (Jux et al. 1998). These data suggest therefore that IGFs are important bone formation stimulators and the inhibitory effects of glucocorticoid on bone formation (e.g. collagen synthesis) may in part be mediated via decreased bone cell production of IGFs. Glucocorticoid treatment also decreases bone cell production of IGF-binding proteins, which have been shown to modulate IGF actions in osteoblasts (Chevalley et al. 1996). Because bone cell production of stimulatory IGF-binding proteins is upregulated by GH, the present study was performed to assess whether the inhibitory effects of glucocorticoids on bone formation and on collagen synthesis can be overcome by treatment with GH and whether a combination of GH plus IGF-I results in an enhanced effect.

Materials and Methods

Subjects

Written informed consent to participate was obtained from 24 healthy male volunteers, aged 24·5 ± 1·2 years and with a body mass index of 23·1 ± 0·6 kg/m². They had no
abnormalities on physical examination or on routine chemical and hematological laboratory tests and were without family history of diabetes mellitus or gastric ulcer disease. They were on no medication during the study protocol. The protocol was reviewed and approved by the ethical committee of the University Hospital Basel.

Protocol

All subjects received methylprednisolone (Urbason, Hoechst, Frankfurt, Germany; 0·5 mg/kg per day) for 6 days (morning of day 1 until morning of day 7), divided in three equal daily doses taken orally together with the main meals, except for the last 18 h of the study (1800 h of day 6 until 1200 h of day 7) when the same dose of methylprednisolone (Urbason Soluble, Hoechst) was infused i.v. and the subjects were hospitalized overnight. In addition to methylprednisolone treatment the subjects were randomly allocated into one of three groups receiving in a double blind fashion either: recombinant human GH (Genotropin) 2 × 0·15 IU/kg per day s.c. (n=8); rhGH at the same dose plus rhIGF-I (Igef) 2 × 40 µg/kg per day s.c. (n=8); or placebo (0·9% NaCl s.c., n=8). Genotropin and Igef were kindly donated by Pharmacia & Upjohn AB, Duebendorf, Switzerland. All injections were administered by a physician (K B) at 0800 h and at 2000 h; the last injections were given at 0800 h on day 7. In the mornings of day 1 (before treatment) and of day 7, blood and urine samples were obtained from the subjects after an overnight fast and after a gelatine-free standardized dinner the evening before. Urine was collected from 0600 h to 1200 h while the subjects remained in the metabolic ward.

Analytical methods

Plasma calcium was measured using a complexometric method, plasma phosphorus and creatinine enzymatically, alkaline phosphatase (ALP) according to the International Federation of Clinical Chemistry at 37 °C, urinary calcium by atomic absorptiometry, and urinary creatinine by a colorimetric assay (Woessner 1961, Podenphant 1984) and urinary pyridinoline and deoxypyridinoline by reverse-phase ion-paired HPLC (Seibel et al. 1989, Black et al. 1989). Serum total IGF-I and GH were measured as described previously (Oehri et al. 1996).

Statistical analyses

Results are expressed as means ± s.e.m. Repeated measures ANOVA of Statview and Student’s paired and unpaired t-tests (Abacus Concepts Inc., Berkeley, CA, USA) on a Power Macintosh 7100/80 were used to detect differences between and within the three protocols. Bonferroni/Dunn and Scheffe’s F procedures were performed for correction of multiple comparisons.

Results

Bone and connective tissue formation

Serum osteocalcin was 32·9 ± 2·1 µg/ml before and decreased to 9·0 ± 1·4 µg/l after 6 days of methylprednisolone treatment in the placebo group (P<0·0001 vs day 1), compared with 36·6 ± 4·3 µg/ml before and 14·8 ± 2·3 after methylprednisolone (P<0·006) in the GH group and 38·8 ± 4·0 µg/ml before and 14·9 ± 2·2 after methylprednisolone in the GH+IGF-I group (P<0·005) respectively (Fig. 1). The decreases were not significantly different between the three groups. Serum PICP decreased during methylprednisolone treatment in the placebo group by 52 ± 7% (P<0·005). The decrease of PICP was slightly less (NS) in the GH alone group, and blunted in the combination group (P<0·005 vs placebo, P<0·05 vs GH). Similarly, serum PIIINP concentrations decreased in the placebo group during methylprednisolone treatment by 37 ± 5% (P<0·005), whereas the decrease of PIIINP during GH+IGF-I treatment was almost completely abolished (P<0·005 vs placebo).

Bone resorption

Both urinary excretion of pyridinoline and deoxypyridinoline increased during methylprednisolone treatment in all three groups (P<0·05 or less) with no significant effect of either GH or GH+IGF-I (Fig. 1B). The increase in hydroxyproline/creatine excretion during methylprednisolone treatment was enhanced by treatment with GH (P<0·005 vs placebo) and even more by combined GH+IGF-I administration (P<0·005 vs placebo, P<0·05 vs GH alone).

GH or GH+IGF-I had no significant effect on urinary creatinine excretion in overnight samples compared with those from the methylprednisolone alone group (placebo group 16·6 ± 0·8 and 16·7 ± 0·08 mmol/l, GH group 14·9 ± 0·9 and 15·1 ± 0·9 mmol/l, GH+IGF-I group 15·4 ± 0·6 and 15·7 ± 0·6 mmol/l on days 1 and 7 respectively).
Concentrations of serum PTH, plasma ALP, bone-specific ALP, plasma calcium and inorganic phosphorus (Pi)

As shown in Table 1, serum PTH increased during methylprednisolone treatment in the placebo group (P<0.05 vs day 1, P<0.05 vs GH+IGF-I group), and remained unchanged in the other two groups. Plasma ALP remained unchanged in the placebo group and decreased during GH treatment (P<0.05 vs day 1) and during GH+IGF-I treatment (P<0.005 vs day 1). Bone-specific ALP decreased during GH treatment (P<0.05 vs day 1). Plasma Pi did not change significantly in any group. Plasma Pi increased in the GH (P<0.005 vs day 1) and in the GH+IGF-I group (P<0.005 vs day 1) but was not changed significantly in the placebo group (P=0.08 vs day 1).

Serum GH and IGF-I concentrations

Serum GH concentrations increased in the GH group from 0.64 ± 0.30 µg/l on day 1 to 21.0 ± 2.2 µg/l on day 7 (P<0.0001 vs placebo) and in the GH+IGF-I group from 0.72 ± 0.30 to 19.5 ± 1.8 µg/l (P<0.0001 vs placebo) with no difference between the GH and the combination group. Total serum IGF-I concentrations on day 1 were 156 ± 12, 203 ± 16 and 198 ± 19 ng/ml in the placebo, GH and GH+IGF-I groups respectively.
They increased modestly on day 7 to 209 ± 16 in the placebo group, to 837 ± 65 in the GH group (P<0·0001 vs placebo) and to 1521 ± 116 ng/ml in the GH+IGF-I group (P<0·005 vs GH).

Discussion

The findings of this study demonstrate that short-term treatment with moderate doses of methylprednisolone (approximately 30 mg/day) decreases osteoblast activity and collagen synthesis and increases bone resorption, in agreement with previous studies (Reid 1989, Lukert & Raisz 1990, Oikarinen et al. 1992). The combination of GH and IGF-I, but not GH alone, was able to inhibit the effects of glucocorticoids on bone collagen formation (as assessed by serum PICP concentrations) and on soft tissue collagen synthesis (as assessed by serum PIINP levels). The glucocorticoid-induced decrease of collagen synthesis was blunted during treatment with IGF-I since serum PICP decreased less in the combination group than during GH treatment alone; GH alone did not affect parameters of collagen synthesis.

The observation that GH and IGF-I had no significant effect on serum osteocalcin concentration, a specific marker of osteoblast activity (Riggs et al. 1986), was unexpected. Osteoblasts express GH and IGF-I receptors, and GH and IGF-I have been demonstrated to exert synergistic proliferative effects on human osteoblasts in vitro (Langdahl et al. 1998). There are several explanations why IGF-I or GH alone failed to affect serum osteocalcin concentrations during glucocorticoid treatment. First, there may have been diminished local responsiveness of osteoblasts to GH and IGF-I during glucocorticoid therapy (Delany et al. 1994). Secondly, glucocorticoids may have altered the production of IGF-binding proteins, thereby attenuating IGF-I effects, e.g. by affecting the stimulating binding protein 4 or the inhibiting binding protein 5 (Lukert & Raisz 1990, McCarthy et al. 1990, 1994, Centrella et al. 1993). Thirdly, a somatomedin inhibitor (Unterman & Phillips 1985) or a factor causing proteolysis of IGF-I or its binding proteins (Conover et al. 1993a,b, Durham et al. 1994, Kamyar et al. 1994) may have been induced by glucocorticoid treatment. Fourthly, direct effects of glucocorticoids (Lukert & Raisz 1990) on osteoblast function independently of GH/IGF-I may have played a role in glucocorticoid-induced bone loss. However, these points do not explain the different responses of serum osteocalcin and PICP concentrations; this discrepancy may be better explained by the finding that glucocorticoids have a direct effect on osteocalcin promoter and decrease osteocalcin gene expression (Aslam et al. 1995, Meyer et al. 1997). Thus, serum osteocalcin levels may not accurately reflect bone formation in patients receiving glucocorticoids but rather reflect the inhibitory effect of glucocorticoids on osteocalcin gene expression. Serum levels of type I collagen may be a better reflection of bone formation in glucocorticoid-treated subjects since glucocorticoids have not been shown to directly affect collagen promotion. The changes in total and skeletal ALP were small and only significantly reduced in the GH and GH+IGF-I group, but not in the placebo group. This discrepancy between ALP and the other parameters of bone formation might be due to several factors: biovariability, small study group and particularly the longer half-life of ALP (around 40 h) compared with PICP in view of a relatively short study period (Crofton 1982).

The glucocorticoid-induced increase in bone resorption was not affected by GH or by the combination of GH plus IGF-I, as indicated by unchanged urinary pyridinoline and deoxypyridinoline excretion. However, whole body collagen breakdown as assessed by urinary hydroxyproline excretion was enhanced during both GH and GH+IGF-I administration when compared with the placebo group.

In contrast to the present findings it has recently been demonstrated in osteoporotic subjects that treatment with either IGF-I or GH enhanced both bone formation and bone resorption (Johansson et al. 1996). Similarly, normal subjects demonstrated an increase in bone turnover during treatment with IGF-I and GH and combined administration of IGF-I and GH synergized in this effect (Mauras et al. 1999).

Table 1 Concentrations of serum PTH, plasma ALP, bone-specific ALP, plasma calcium and plasma Pi

<table>
<thead>
<tr>
<th></th>
<th>PTH (pg/ml)</th>
<th>ALP (U/l)</th>
<th>Bone-specific ALP (U/l)</th>
<th>Calcium (mmol/l)</th>
<th>Pi (mmol/l)</th>
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</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>21·2±3·2</td>
<td>21·4±1·8</td>
<td>13·3±1·8</td>
<td>2·32±0·02</td>
<td>1·11±0·03</td>
</tr>
<tr>
<td>GH</td>
<td>16·2±2·9</td>
<td>19·8±1·8</td>
<td>16·3±3·7</td>
<td>2·38±0·01</td>
<td>1·10±0·03</td>
</tr>
<tr>
<td>GH+IGF-I</td>
<td>24·0±2·4</td>
<td>21·9±2·9</td>
<td>11·3±3·3</td>
<td>2·40±0·02</td>
<td>1·09±0·04</td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>27·4±4·6</td>
<td>18·6±0·8</td>
<td>13·2±1·7</td>
<td>2·34±0·02</td>
<td>1·24±0·05</td>
</tr>
<tr>
<td>GH</td>
<td>22·7±5·7</td>
<td>18·0±1·9*</td>
<td>13·4±4·0*</td>
<td>2·43±0·03</td>
<td>1·31±0·05**</td>
</tr>
<tr>
<td>GH+IGF-I</td>
<td>23·7±1·8‡</td>
<td>18·0±2·5**</td>
<td>7·3±2·3</td>
<td>2·38±0·02</td>
<td>1·20±0·04**</td>
</tr>
</tbody>
</table>

*P<0·05 vs day 1, **P<0·005 vs day 1, ‡P<0·05 vs placebo (ANOVA).
et al. 1996). GH increased markers of bone turnover in elderly women, with an increase in hydroxyproline and pyridinoline excretion and an increase in plasma osteocalcin concentrations (Holloway et al. 1994). However, GH failed to prevent cortical osteopenia induced by glucocorticoids in rats (Ortoft et al. 1995).

These findings indicate that GH and/or IGF-I treatment increase bone turnover in humans only under certain conditions. A stimulatory effect may not be observed during glucocorticoid treatment. In agreement with this conclusion are data recently obtained in our laboratory. We assessed the effects of IGF-I alone in eight normal subjects on parameters of bone turnover during glucocorticoid administration in a protocol identical to that described in this study (M Oehri, M Kraenzlin, K Berneis & U Keller, unpublished data). We observed that IGF-I alone did not affect any of the glucocorticoid-induced changes of bone turnover.

The increase in serum PTH levels observed in the present study during treatment with glucocorticoids was abolished by co-administration of GH plus IGF-I. Increased PTH levels during glucocorticoid administration have been demonstrated previously (Hahn et al. 1979, Suzuki et al. 1983).

Therefore, based on the present findings it is concluded that glucocorticoids exert profound effects on markers of bone turnover, such as diminished osteoblast function and increased bone resorption. Combined therapy with GH and IGF-I counteracts the decrease of bone and soft tissue collagen synthesis but does not affect parameters of bone resorption. The findings indicate that combined treatment with GH and IGF-I may be more effective than GH alone in preventing diminished collagen synthesis in patients receiving glucocorticoid treatment.

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