Insulin-Like Growth Factor I Enhances the Formation of Type I Collagen in Hydrocortisone-Treated Human Osteoblasts

Kenneth B. Jonsson,1,3 Sverker Ljunghall,1 Olle Karlström,1 Anna G. Johansson,1 Hans Mallmin,2 and Östen Ljunggren1

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We have studied the effect of insulin-like growth factor I (IGF-I) on the formation of osteocalcin and type I collagen in isolated human osteoblasts. IGF-I at and above 0.1 nM stimulated the formation of type I collagen as measured by the type I procollagen carboxyterminal peptide (PICP), in human osteoblasts, incubated for 72 hrs in serumfree conditions. The secretion of osteocalcin was not affected by IGF-I while 1,25(OH)2vitamin D3 significantly enhanced the formation of osteocalcin. When human osteoblast-like cells were incubated with hydrocortisone (1 µM), a significant decrease in the release of both PICP and osteocalcin was seen. Addition of IGF-I to human osteoblasts also treated with hydrocortisone normalized the PICP-formation but did not affect the suppressed osteocalcin-formation. These data indicate that IGF-I reverses selective effects of hydrocortisone on bone.

KEY WORDS: bone; glucocorticoids; insulin-like growth factor I osteoblasts; collagen; osteocalcin.

INTRODUCTION

Glucocorticoids have complex effects on bone and mineral metabolism. When present in excess, they are known to cause osteoporosis (1–4). This side-effect of treatment with glucocorticoids has been attributed to an impaired function of the osteoblasts, as well as to increased bone resorption (3, 4). The direct effects of glucocorticoids on the osteoblast are not fully understood. Cortisol seems to have a biphasic action on cell growth and collagen synthesis in bone organ culture (5). In several experimental systems, collagen synthesis is either increased or decreased, depending on steroid concentration and duration of treatment. However, it seems plausible that the dysfunction of osteoblasts seen in cultures with supraphysiological steroid concentrations may be important in the pathogenesis of glucocorticoid-induced osteoporosis (3, 4, 6). Skeletal cells have been

1 Department of Internal Medicine and 2 Department of Orthopaedic Surgery, University Hospital, S-751 85 Uppsala, Sweden.
3 To whom correspondence should be addressed.
shown to synthesize insulin-like growth factor I (IGF-I) (7, 8), a polypeptide
growth factor known to enhance proliferation of osteoblastic cells and to
stimulate synthesis of bone specific proteins in cultures of osteoblasts and bone
(9–11). The local production of IGF-I in bone is under hormonal control
(8, 12, 13), and studies indicate that glucocorticoids may play an important role in
the regulation of the local production of IGF-I (14–16). Thus, it is possible that
decreased IGF-I synthesis is involved in steroid-induced inhibition of anabolic
osteoblastic function.

In this report, we describe an investigation of the effects of IGF-I on
hydrocortisone-treated isolated human bone cells.

MATERIALS AND METHODS

Materials

Eagle’s minimal essential medium, alpha modification (α-MEM) was pur-
chased from Northumbra Biological Ltd, Cramlington, UK; penicillin and
streptomycin (PEST), L-glutamine, trypsin-EDTA, and fetal calf serum (FCS)
from Biochrom KG, Berlin, Germany; human parathyroid hormone fragment
1–34 (PTH), bovine serum albumin (BSA) and hydrocortisone from Sigma
Chemical Co., St. Louis, MO, USA. Gibco CMRL-1066 medium was from Life
Technologies Ltd, Paisley, Scotland, UK. 1,25(OH)2vitamin D3 was kindly
provided by LEO, Ballerup, Denmark, and recombinant human IGF-I by Kabi
Pharmacia AB, Stockholm, Sweden. The commercial RIA-kit for human type I
Procollagen carboxyterminal peptide (PICP) was from Farmos Diagnostica,
Turku, Finland, and the commercial RIA-kit for human osteocalcin was from
CEA, Oris, France.

Isolation of Human Osteoblast-like Cells

Trabecular bone was obtained from the iliac crest used as donor site in
patients undergoing bone graft procedures. The specimens were cut into small
fragments, 1–2 mm in diameter, thoroughly rinsed with phosphate-buffered
saline, and cultured in 78 cm² tissue culture dishes containing α-MEM supple-
mented with PEST (100 U/ml of penicillin, 100 µg/ml of streptomycin) 2 µM
L-glutamine and 10% FCS. After 3–4 weeks, the culture dishes were confluent
with cells that had migrated from the trabecular bone. The cells were detached
with trypsin-EDTA (0.05/0.02% w/v), and seeded into 2 cm² multiwell culture
dishes in which the subsequent experiments were performed when the cells were
confluent. The human bone-derived cells obtained by this procedure respond in a
dose-dependent manner to PTH with increasing amounts of intracellular cyclic
AMP (data not shown), to 1,25(OH)2vitamin D3 with increased osteocalcin
synthesis, and have spontaneous collagen type I release as measured by PICP.
Measurement of Osteocalcin and PICP Formation

Human osteoblast-like cells were cultured in 96-well plastic culture dishes in CMRL-1066 culture medium supplemented with PEST and L-glutamine in concentrations as above, and BSA (1 mg/ml). Test substances (IGF-I and hydrocortisone) were added together at the beginning of culture. After 72 hrs the medium was collected and the amounts of osteocalcin and PICP in the supernatants were analysed by RIA. After harvesting, the number of osteoblast-like cells per well was counted in a Bürker chamber and the amounts of the two proteins, measured by RIA, were calculated per 1000 cells.

RESULTS

IGF-I at and above 0.1 nM stimulated the formation of PICP in human osteoblasts, incubated for 72 hrs in serumfree conditions (Fig. 1a). The formation of osteocalcin was not affected by IGF-I while 1,25(OH)₂vitamin D₃ (10 nM) significantly enhanced the formation of osteocalcin (Fig. 1b). When human bone cells were incubated with 1,25(OH)₂vitamin D₃ (10 nM), addition of hydrocortisone (1 μM) significantly decreased the formation of both PICP and osteocalcin (Table 1). Addition of IGF-I to these cultures reversed the inhibitory effect on PICP-formation but did not affect the suppressed osteocalcin formation (Table 1). A time course study showed that hydrocortisone (1 μM) inhibited the formation of PICP after 24 hrs incubation. Addition of IGF-I (0.1 μM) reversed the inhibitory effect of hydrocortisone after 48 hrs and onwards (Fig. 2).

DISCUSSION

Treatment with glucocorticoids causes osteopenia. It is believed that cortisone-induced osteoporosis is partly due to a direct inhibitory effect on the osteoblasts (3, 4). In this report we have used isolated human bone cells and we show that IGF-I has selective anabolic effects in these cells, as indicated by increased collagen synthesis, and that IGF-I reverses some of the effects of hydrocortisone on bone metabolism.

In vivo it is known that treatment with cortisone causes a rapid decrease in serum levels of osteocalcin (14). It was previously reported that addition of IGF-I to human osteoblast-like cells, simultaneously treated with 1,25(OH)₂vitamin D₃, inconsistently stimulated osteocalcin synthesis in the presence of fetal bovine serum (8). In a male osteoporotic patient treated with IGF-I osteocalcin serum levels increased (15). Paradoxically, in this study we did not detect any response to stimulation of IGF-I on osteocalcin synthesis and we conclude that in serumfree conditions IGF-I does not affect osteocalcin synthesis in human osteoblast-like cells.

Cortisone in high concentrations inhibits the formation of bone specific proteins in cultured osteoblasts (3–6). It is possible that this could be secondary
Fig. 1. Effects of IGF-I on the formation of PICP and osteocalcin in human osteoblasts. Human osteoblast-like cells were isolated from trabecular bone as described in methods. The cells were seeded into 2 cm² plastic multiwell culture dishes and cultured for 72 h in CMRL-1066 culture medium with antibiotics and BSA. The amounts of PICP and osteocalcin in the culture media were analysed by RIA at the end of the experiments. The figure shows pooled data from two separate experiments with five wells to each group. Basal formation of osteocalcin was 3.6 ± 0.2 and 3.6 ± 0.4 ng/1000 cells (means ± SEM). Basal formation of PICP was 1360 ± 80 and 1160 ± 30 μg/1000 cells (means ± SEM). ■ = 1,25(OH)₂vitamin D₃ (10 nM).
Table 1. Effect of IGF-I on hydrocortisone treated human osteoblasts. Human osteoblast-like cells were isolated from trabecular bone as described in methods. The cells were seeded into 2 cm² plastic multiwell culture dishes and cultured for 72 h in CMRL-1066 culture medium with antibiotics and BSA. The cells were challenged with 1,25(OH)₂vitamin D₃ (0.1 nM), hydrocortisone (HC, 1 μM), and IGF-I (0.1 μM) as shown in the table. The substances were added together at the beginning of culture. The concentration of PICP and osteocalcin in the collected supernatants were determined by RIA at the end of the experiments. Similar results were obtained in three independent experiments.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Osteocalcin (pg/1000 cells)</th>
<th>PICP (ng/1000 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1,25(OH)₂vitamin D₃</td>
<td>134 ± 4</td>
<td>—</td>
</tr>
<tr>
<td>—— + HC</td>
<td>241 ± 13ᵃ</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>—— + IGF-I</td>
<td>154 ± 15ᵇ</td>
<td>32 ± 3ᵇ</td>
</tr>
<tr>
<td>—— + —— + IGF-I</td>
<td>153 ± 6ᵇ</td>
<td>46 ± 4</td>
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</tbody>
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Values represent means ±SEM for 5 wells.
ᵃ Significantly different from control, p < 0.01.
ᵇ —— 1,25(OH)₂vitamin D₃-treated control, p < 0.01.

to inhibition of local growth factor synthesis in the bone. IGF-I has known anabolic effects on bone and diminished production of IGF-I in fetal rat calvariae and rat osteoblast-like cells treated with cortisone suggests a possible mechanism of action in this type of osteopenia (16, 17). However, glucocorticoids transiently increase collagen synthesis in vitro and also increase the response to exogenous IGF-I (3, 4, 18). This paradox has so far not been satisfactorily explained. Today’s treatment for osteoporosis is primarily focused on anti-resorptive agents, e.g. oestrogen, bisphosphonates and calcitonin (19). In view of the belief that a dysfunctioning osteoblast is the major cause of cortisone-induced osteoporosis it would be more suitable to develop an anabolic treatment for this type of osteopenia. Our finding that IGF-I is capable of reversing the inhibitory effect of
hydrocortisone on collagen synthesis in cultured human osteoblasts suggests that IGF-I may be a potential candidate.

REFERENCES