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Effect of glucocorticoid treatment on Wnt signalling antagonists (sclerostin and Dkk-1) and their relationship with bone turnover

L. Gifre [a,*], S. Ruiz-Gaspà [b], A. Monegal [a], B. Nomdedeu [c], X. Filella [d], N. Guañabens [a,b], P. Peris [a,b]

[a] Rheumatology Department, Metabolic Bone Diseases Unit, Hospital Clínic of Barcelona, Spain
[b] CIBERehd, Hospital Clinic of Barcelona, Spain
[c] Haematology Department, Hospital Clínic of Barcelona, Spain
[d] Department of Biochemistry and Molecular Genetics, Hospital Clínic of Barcelona, Spain

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ABSTRACT

The aim of this study was to analyse the effect of glucocorticoid therapy (GCCT) on Wnt signalling antagonists (sclerostin and Dkk-1) and their relationship with bone turnover. 25 patients (8 M/17 F, aged 48 ± 19 yrs) recently initiating GCCT (≥7.5 mg/day, ≤6 months) were prospectively included. Bone turnover markers (bone formation: P1NP, osteocalcin [OC], bone ALP; bone resorption: sCTx) and Wnt antagonists (serum sclerostin and Dkk-1) were assessed in all patients (short-term and 12 months after initiating GCCT). Bone mineral density (BMD) was performed to assess osteoporosis. The results were compared with 60 healthy controls. At short-term patients on GCCT showed a significant decrease in bone formation markers versus controls (P1NP: 19 ± 9 vs. 43 ± 16 ng/mL, $p < 0.001$; OC: 7.4 ± 2.4 vs. 18.4 ± 5.2 ng/mL, $p = 0.001$) and in Dkk-1 levels (24.5 ± 20.1 vs. 36.8 ± 13.7 pmol/L, $p = 0.008$) with similar sclerostin values (41.8 ± 21.8 vs. 42.1 ± 13.9 pmol/L, $p = 0.950$). Sclerostin correlated positively with GCCT doses ($r = 0.449$, $p = 0.024$) and lumbar BMD ($r = 0.424$, $p = 0.035$), and negatively with bone ALP ($r = -0.398$, $p = 0.049$). A progressive decrease in Dkk-1 levels was observed at 12 months, (19.1 ± 14.9, $p = 0.001$), whereas sclerostin increased compared to controls (48.9 ± 11.6, $p = 0.045$). In conclusion, the effect of GCCT on the serum levels of the Wnt signalling parameters differs depending on the antagonist evaluated. Whereas sclerostin values increased and showed a relationship with the dose and bone AP, Dkk-1 levels decreased throughout the study suggesting a counter-regulatory mechanism of this factor thereby reducing the deleterious effect of GCCT in the bone.

Introduction

Glucocorticoid (GCC)-induced osteoporosis is one of the most frequent causes of secondary osteoporosis [1,2]. The adverse effects of GCC in the bone are primarily in osteoblastic and osteocyte cells, impairing osteoblast differentiation and function and increasing the apoptosis of osteoblasts and osteocytes [3,4]. In addition, recent data have also indicated an increased autophagy in osteocytes induced by GCC [5]. All these effects lead to suppression of bone formation, a common feature in the pathogenesis of GCC-induced osteoporosis. Indeed, in this process avoidance of osteoblast and osteocyte apoptosis results in preservation of bone strength [6].

The mechanism by which GCC impairs bone formation and osteoblast function seems to be by opposing Wnt/-β-catenin signalling. Wnt signalling and its antagonists (sclerostin and Dickkopf [Dkk-1]) play an important role in the regulation of bone mass and osteoblastogenesis. In this pathway, when Wnt is absent, β-catenin is phosphorylated and degraded, and when Wnt is present it binds to specific receptors (frizzled) and coreceptors (LRP-5 and -6), leading to inhibition of β-catenin phosphorylation and degradation; this results in stabilization and translocation of this molecule to the nucleus where it interacts with transcription factors to regulate gene expression. In addition, Wnt/-β-catenin signalling is not only critical for osteoblastogenesis but also for normal osteocyte function [7]. The binding of Wnt antagonists, sclerostin and Dkk-1, to the canonical Wnt-β-catenin receptors inhibits this signalling and consequently bone formation. These antagonists seem to be mostly synthetized by osteocytes. However, whereas in adults sclerostin is almost exclusively expressed in osteocytes and late osteoblasts [8], Dkk-1 is also expressed in several tissues, such as, endothelial cells, neural cells and platelets, among others, the latter being considered a major contributor to circulating Dkk-1 levels in recent studies [9,10].

It is currently believed that GCC therapy impairs osteoblastic differentiation by inhibiting the canonical Wnt/β-catenin signalling pathway, especially through increased expression of the antagonists of Wnt

Abbreviations: GCCT, glucocorticoid therapy; GCC, glucocorticoid; Dkk-1, Dickkopf; BMD, bone mineral density; SD, standard deviation of the mean; BMI, body mass index; bone ALP, bone alkaline phosphatase; OC, osteocalcin; P1NP, propeptide amino-terminal of type I procollagen; sCTX, serum carboxy-terminal telopeptide of type I collagen.

* Corresponding author at: Service of Rheumatology, Hospital Clínic, 08036 Barcelona, Spain. Fax: +34 932279386.
E-mail address: lgifre@clinic.ub.es (L. Gifre).

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signalling, Dkk1 and sclerostin, a finding that has been observed in previous experimental studies in rodents and cell cultures [3,4,11–13].

At present, data on the effects of GCC therapy on Wnt pathway antagonists, sclerostin and Dkk-1 in humans are scarce, with recent data showing a decrease in sclerostin serum levels during the first week of GCC treatment [14] and increased Dkk-1 levels in patients with 21-hydroxylase deficiency chronically supplemented with GCC [15]. Consequently, evaluation of these mediators together with that of bone turnover markers in this population would improve the physiopathological knowledge of the bone loss related to this process, allowing a better therapeutic approach in these patients.

Therefore the aims of the present study were: to analyse the serum levels of sclerostin and Dkk-1 in patients with recent onset of GCC treatment and compare these levels with those of healthy controls of similar age and gender; to analyse the relationship of these antagonists with bone turnover markers and bone mineral density (BMD) in these patients, and to analyse the long-term evolution of sclerostin and Dkk-1 and their relation with bone turnover markers.

Patients and methods

Patients and study design

A total of 30 patients were prospectively evaluated for the study. Of these 25 subjects (8 males/17 females [9 postmenopausal]) aged 17 to 84 years (mean ± SD age, 48 ± 19 years) met the inclusion criteria and were included in the study. All subjects had recent onset of GCC treatment (less than 6 months) at doses over 7.5 mg per day. Patients were recruited from the Haematology Department, from June 2010 to June 2012. Except for GCC therapy, none had an evident secondary cause of osteoporosis (i.e., malabsorption or known endocrine disorders associated with bone loss) nor was any patient receiving antiosteoporotic treatment at the time of inclusion in the study. Visits and evaluations, including biochemical determinations, were performed at short-term (shortly after the onset of GCC treatment) and at 12 months. We also analysed lumbar and femoral BMD at baseline.

In all patients, risk factors for osteoporosis were assessed, including: history of fragility fractures, tobacco and alcohol consumption (considered as ≥2 drinks per day in women and ≥3 in men), body mass index (BMI), mean dietary calcium intake (mg/day), associated comorbidities and, in women, menopausal status, among others. In addition, the cause for GCC treatment, GCC doses (daily [mg/day] and cumulative [grammes]) as well as the duration of GCC treatment (days) were recorded in all patients.

The results were compared with a control group constituted by 60 healthy subjects (18 males/42 females [20 postmenopausal]) of similar age (44 ± 15 years, range 22–80) and gender with no evidence of disturbances of calcium metabolism or metabolic bone disease.

All patients provided informed consent to participate and the Ethics Committee of the hospital approved the study.

Biochemical determinations

Blood samples were obtained between 8:00 and 10:00 a.m. after an overnight fast. Serum samples were aliquoted and kept frozen at −80 °C until analysis.

The measurements included: bone alkaline phosphatase [bone ALP], osteocalcin [OC] and propeptide amino-terminal of type I procollagen [P1NP] as serum bone formation markers, assessed by ELISA (IDS, Vitro), IRMA (Elsa-Osteo-Cis, Gif-sur-Yvette, France) and electrochemiluminescence by the automated method Cobas e411 (Roche), respectively. Serum carboxy-terminal telopeptide of type I collagen [sCTX] (automated method Cobas e411, Roche) was determined as a bone resorption marker. The intra-assay and inter-assay coefficients of variation for each marker were as follows: bone ALP 2.9% and 5.8%; P1NP 2.8% and 4.3%, OC 4.1% and 3.5%, and sCTX 2% and 5.7%.

Serum sclerostin and Dkk-1 were assayed in duplicate using an ELISA kit (Biomedica Medizinprodukte GmbH & Co. KG, Wien, Austria). The intra-assay and inter-assay coefficients of variation were as follows: sclerostin 4–6% and 5–7%; and Dkk-1 7–8% and 9–12%.

Bone mineral density measurements

Bone mineral density (BMD) of the lumbar spine (L2–L4), femoral neck and total hip was measured by dual X-ray absorptiometry (Lunar Prodigy, Radiation Corporation Madison, WI). BMD risk categories were defined as normal, osteopenia or osteoporosis, based on current WHO T-score definitions [16].

Statistical analysis

All data are expressed as mean ± SD (standard deviation of the mean). The Kolmogorov–Smirnov test was used to assess the normal distribution of the variables. The nonparametric Kruskal–Wallis or Mann–Whitney tests were used to compare differences for continuous variables. Differences between proportions were assessed by the Chi square test. The Wilcoxon test was used to assess differences among GCC paired samples. A p value of <0.05 was considered statistically significant.

Statistical analyses were carried out using SPSS version 18.0®.

Results

The clinical and biochemical characteristics of the patients are included in Table 1.

Most patients were included during the first 2 months (80%) of GCC treatment (mean 49 ± 31 days, range 4–127) with a mean daily GCC dose of 64 ± 16 mg/day of prednisone and a total GCC dose received of 2.86 ± 1.32 g at the time of inclusion. Idiopathic thrombocytopenic purpura (ITP) (76%) and haemolytic anaemia (12%) were the most frequently associated conditions.

Eight patients were lost to follow-up, thus 15 of the 25 patients underwent follow-up at 12 months. The causes for this loss of follow-up in GCC patients were: 8 patients did not attend the control follow-

Table 1
Clinical characteristics of the patients included in the study.

	Patients on GCC therapy (n = 25)
Age (years)	48 ± 19 (17–84)
Sex/menopausal status (n)	
Men	8
Women (postmenopausal)	17 (9)
Risk factors for osteoporosis	
BMI (kg/m^2)	25.9 ± 5.2
Dietary calcium intake (mg/day)	583 ± 287
Current tobacco consumption (%)	16
Current alcohol consumption (%)	8
History of fragility fractures (%)	0
GCC-requiring illness (%)	
Idiopathic thrombocytopenic purpura	76
Haemolytic anaemia	12
Other causes	12
GCC dose and duration	
Daily GCC doses (mg/day)	64 ± 16
Duration of GCC treatment (days)	49 ± 31
Cumulative GCC doses (g)	2.86 ± 1.32
BMD T score measurements at baseline	
Lumbar	−0.77 ± 1.33
Femoral neck	−0.53 ± 1.15
Total hip	−0.45 ± 1.21

The results are expressed as mean ± SD, % or n.
BMI: body mass index. GCC: glucocorticoid. BMD: bone mineral density.