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Blood sclerostin and Dkk-1 in patients who start treatment with glucocorticoids. Preliminary results

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Summary

Background and objectives: The Wnt pathway and its inhibitors (sclerostin and Dkk-1) have a primary role in the regulation of bone mass and osteoblastogenesis. The objective of this study was to analyse the effect of treatment with glucocorticoids (GCC) on the inhibitors of the Wnt pathway and their relationship with bone mass and the parameters for bone turnover.
Methods: A transverse study including 15 patients (9 women and 6 men) with an mean age of 51±21 years at the start of treatment with GCC (≥7.5 mg/day, ≤6 months). Levels of sclerostin, blood Dkk-1 and blood markers for bone turnover (procollagen 1 N-terminal propeptide [P1NP], osteocalcin [OC], and carboxy-terminal telopeptide of collagen type 1 [CTX]) were determined, and bone densitometry (DXA) in the lumbar spine was carried out, in all patients. The results were compared with a control group.
Results: The mean dose of glucocorticoids was 58±21 mg/day, in the majority of patients (73%) indicated by idiopathic thrombocytopenic purpura. The patients treated with glucocorticoids had a reduction in the parameters for bone formation compared with a control group (OC: 7.4±2.8 vs 24.4±6.2 ng/ml, $p<0.01$) and a reduction in blood Dkk-1 (29.6±23.6 vs 48.3±15.6 pmol/L, $p=0.02$). No significant differences were observed in values for blood sclerostin, although this correlated positively with the dose of GCC received and lumbar bone mineral density.
Conclusion: Contrary to what is seen in experimental studies, the start of treatment with glucocorticoids is associated with a reduction in blood levels of Dkk-1. These results indicate the necessity of analysing these inhibitors and their relationship with remodelling and bone mass during this process over the long term.

Key words: *sclerostin, Dkk-1, glucocorticoids.*

Introduction

Treatment with glucocorticoids (GCC) is associated with a marked loss of bone mass and the development of fractures in the initial phases of treatment, as well as being one of the most common causes of secondary osteoporosis[1]. The GCCs act especially on the osteoblasts and osteocytes, reducing the replication, differentiation and function of the osteoblasts and inducing apoptosis in the osteoblasts and osteocytes. These changes lead over time to a reduction in the formation and quality of bone, which is the finding most characteristic of secondary osteoporosis induced by GCC[2-4].

The Wnt pathway, a cell signalling pathway, has a fundamental role in the modulation of osteoblast activity. This pathway is integrated through various components which include ligands, membrane receptors, intracellular effectors and antagonists. The Wnt pathway antagonists, notable among which are sclerostin and Dkk-1, bond with the membrane receptors (essentially LRP-5 and -6) and inhibit the activity of this pathway, and consequently osteoblast activity.

Recent experimental studies, both *in vitro* and *in vivo*, indicate that treatment with GCC reduces the differentiation of the osteoblasts through the Wnt pathway by means of an increase in its inhibitors, sclerostin and Dkk-1[5-7]. However, currently there are hardly any clinical data on the effect of treatment with such inhibitors in GCC. Thus the objective of this study has been to analyse the blood levels of sclerostin and Dkk-1 in patients who have recently begun treatment with GCC, and evaluate its relationship with the markers for bone remodelling and bone mineral density (BMD).

Patients and methods

Study population

A transverse study which included patients who had initiated (<3 months) treatment with doses equal to, or greater than, 7.5 mg/day of prednisone or equivalent. The patients were proposed by the haematology service of the Hospital Clinic Barcelona (August 2010 to 2012) and recruited consecutively.

All the patients complied with the following inclusion criteria: aged over 18 and with normal values of creatinine, liver function, calcium and phosphorus. Excluded were patients who had followed treatment with GCC for more than 6 months, those with diseases or processes which affected bone metabolism (Paget's disease, rheumatoid arthritis, hyperparathyroidism, hypercortisolism, malabsorption syndrome, malignant tumours, transplant, pregnancy or recent breastfeeding) and/or those who followed treatment with drugs which interfered with bone metabolism (bisphosphonates, strontium ranelate, selective estrogen receptor modulators, calcitonin, estrogen therapy, denosumab, osteoformers thiazides or anti-convulsives).

In all the patients the risk factors for osteoporosis were evaluated, including: family history of femoral fracture, personal history of fractures, tobacco and alcohol consumption, age at menopause, dietary intake of calcium (mg/day) and history of renal lithiasis. Recorded in addition was the cause of treatment with GCC, the dose and treatment regime (accumulated dose [mg] and duration [days]).

The results were compared with a healthy control group of similar age and sex.

The study was carried out with the approval of the ethics committee of the hospital and conformed with the directives pertinent to research in humans. All the patients signed their informed consent to their inclusion.

Analytical tests

Blood was taken from all patients at between 8 and 10 am after a night of fasting. A biochemical profile was carried out which included calcium, phosphorus, creatinine, and total alkaline phosphatase, determined by standard techniques.

The following biochemical markers for formation were measured: osteocalcin (OC, radioimmunoassay, Elsa-Osteo-Cis, Gif-sur-Yvette, France) and amino-terminal propeptide of procollagen type I (PINP, automated method Cobas e411, Roche); and of bone resorption: carboxy-terminal telopeptide of collagen type I (CTX, automated method Cobas e411, Roche).

Blood levels of sclerostin and Dkk-1 were measured using ELISA (Biomedica, Austria), with a coefficient of intravariation of 4-6% and 7-8%, and a coefficient of intervariation of 5-7% and 9-12%, respectively.

Bone mineral density

The BMD in the lumbar spine and femur were determined in all patients using dual X-ray absorptiometry (DXA; Lunar Prodigy, Radiation Corporation Madison, WI, U.S.). The densitometric risk categories (normal BMD, osteopenia and/or osteoporosis) were defined according to WHO criteria[8].

Statistical analysis

The results have been expressed as the mean ± standard deviation from the mean (SD). The differences between the means of the continuous variables were analysed using the Mann-Whitney non-parametric U test, and the differences between proportions by means of the Fisher test. To evaluate the association between variables the Pearson coefficient of correlation was used. Values $p<0.05$ were considered statistically significant. The statistical analysis of the data was carried out using the SPSS software programme (version 18.0, Chicago, U.S.).

Results

The clinical characteristics of the patients included in the study are shown in Table 1.

15 patients were included (9 women [4 postmenopausal] and 6 men), with an average age of 51±21 years. The average dose of GCC used was 58±21 mg/day (range 20-100 mg/day) and the average duration of treatment was 42±24 days (range 4-90 days). 73% of the patients had received treatment for idiopathic thrombocytopenic purpura, 20% for haemolytic anaemia and 3% for both causes (Evans

syndrome). 27% had family history of fracture of the femur and 20% had densitometric osteoporosis. Dietary intake of calcium was 593±305 mg/day and only one patient was an active smoker. The patients who had followed treatment with GCC showed a significant reduction in markers for formation with respect to the control group (PINP: 18±9 vs 47±9 ng/ml, p<0.01; OC: 7.4±2.8 vs 24.4±6.2 ng/ml, p<0.01). No significant differences were reported in values of markers for resorption with respect to the control group (CTX: 0.60±0.27 vs 0.45±0.16 ng/ml, p=0.21).

Those patients treated with GCC had a reduction in blood levels of Dkk-1 compared with the control group (29.6±23.6 vs 48.3±15.6 pmol/L, p=0.02), while concentrations of sclerostin were similar in both groups (39.6±23.3 vs 33.8±20.3, p=0.6) (Figure 1).

In the group of patients treated with GCC, the values of sclerostin were positively correlated with the accumulated dose of GCC (r=0.573 p=0.026) and lumbar BMD (r=0.550, p=0.034) (Figure 2). The values of Dkk-1 were not related with any of the parameters analysed. No relationship was observed between the values for markers for bone remodelling and blood values of sclerostin and/or Dkk-1. In the control group, the values of sclerostin were positively correlated with age (r=0.661, p=0.02) (Table 2).

Table 1. Clinical characteristics of patients treated with GCC

	Patients with GCC (n=15)
Age (years)	51±21
Sex (female/male)	9/6
Risk factors for osteoporosis:	
BMI (kg/m^2)	25±5
Dietary calcium intake (mg/day)	593±305
History of kidney stones (%)	13
Active smoking (%)	7
Alcohol consumption habitual (%)	13
Family history:	
Femoral fracture (%)	27
Treatment regimen with GCC:	
Daily dose of GCC (mg/day)	58±21
Duration of treatment with GCC (days)	42±24
Cumulative dose of GCC (g)	2.5±1.3
BMD (g/cm^2):	
Lumbar	1.122±0.156
Femoral neck	0.927±0.113
Total femur	0.958±0.109

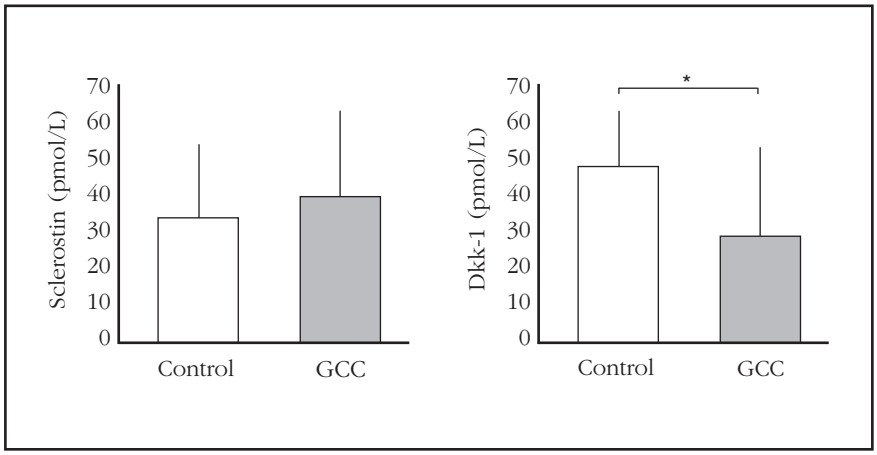
GCC: glucocorticoids; BMI: body mass index; BMD: bone mineral density.

Discussion

Contrary to what occurs in experimental studies, the initiation of treatment with GCC in patients with haematological processes is associated with a reduction in blood levels of Dkk-1. This effect, however, differs as a function of the antagonist analysed, since no significant changes were observed in concentrations of sclerostin at the time of the evaluation.

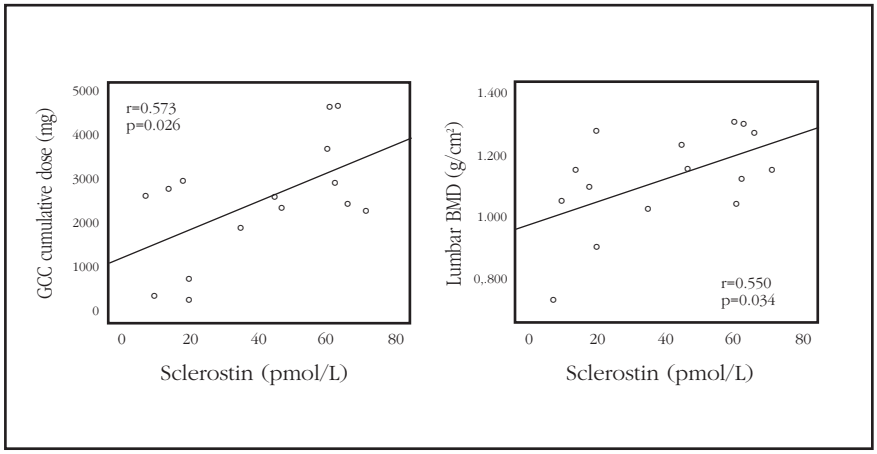
The patients included in this study had low blood levels of Dkk-1 after initiating treatment with GCC at medium-to-high doses. These findings contrasted with the results of earlier experimental studies. Thus, in cultures of osteoblasts and osteocytes (MLO-Y4 cells), treatment with dexamethasone resulted in an increase in Dkk-1[9], which is associated with the dose and period over which treatment is received[7]. There are similar results in animal experimentation models, in which have been observed an increase in the expression of Dkk-1 in the bone tissue after initiating treatment with GCC[7] and an attenuation of the deleterious effect of GCC in the bone by blocking the effect of Dkk-1 in mice[5]. Even though the causes of these differences are not clear, the method of treatment with GCC, including the dose and period of treatment, may explain, in part, these results[6]. Neither can we discount a counter-regulatory effect of this Wnt pathway antagonist in situations of prolonged exposure to GCCs, or that these blood levels may indicate not only the cell function of the osteoblasts and osteocytes but also the number of cells, which, as is well known, reduces (due to an increase in apoptosis) with treatment with corticoids.

Figure 1 . Blood levels of sclerostin and Dkk-1 in patients in treatment with GCC (grey) in comparison with healthy controls (white)



* p=0.02

Figure 2. Correlation between values of sclerostin, the accumulated dose of GCC (mg) and lumbar bone mass (lumbar BMD)



However, it is worth commenting that there have recently been preliminary indications of a reduction in blood values of Dkk-1 at three months from the start of treatment with GCC[10], and similarly, recent studies have described a paradoxical response of Dkk-1 similar to that observed in our study, in other clinical situations. Thus, contrary to expectations, reduced values of Dkk-1 have been observed in immobilised patients[11], and an increase in blood values of Dkk-1 in patients with primary hyperparathyroidism[12]. Also it has been described a paradoxical response of Dkk-1 after treatment with teriparatide[13] and denosumab[14]. These authors have suggested that there is a relationship between bone remodelling and values of Dkk-1. In any case, it is important to remember that there may be factors which influence blood values of Dkk-1, such as an underlying disease and concomitant treatment, among others, which should be taken into account when analysing the concentrations of this antagonist. In addition, the relationship between blood levels and expression in tissue is controversial[7].

In our study, blood values of sclerostin after the initiation of treatment with GCC were similar to those in the control group. However, a positive correlation was observed between values of sclerostin and the accumulated dose of GCC, suggesting a GCC-dependent effect on this Wnt pathway antagonist. Studies in mice treated with GCC have reported an increase in the expression of sclerostin after treatment[3]. However, in patients who started treatment with GCC (in the first 96 hours) a reduction in values of sclerostin has been reported[15], a finding which has not been observed in postmenopausal women treated with GCC[10], nor in patients with hypercortisolism due to Cushing's syndrome[16], in whom there has been observed an increase in values of sclerostin.

The circulating levels of sclerostin in the general population has been associated with age, sex, estrogenic *status* (postmenopause) and the total quantity of bone mass[17-19], which means that these factors need to be taken into account when its concentration is analysed. So, in the healthy subjects included in our study we observed a positive correlation of levels of sclerostin with age, a finding which was not observed in the group treated with GCC, possibly due to the direct effect of the GCC on the Wnt pathway. In this group of patients it was observed, however, that there was a positive correlation between blood levels of sclerostin and lumbar BMD, a relationship which has also been observed in other studies and which has been attributed to a higher production of sclerostin by the osteocytes, due to the greater quantity of bone[18,20].

The relationship between the markers for bone remodelling and the Wnt pathway antagonists is uncertain and varies in different clinical situations. Thus, García-Martin et al.[21,22] in a group of patients with diabetes mellitus type 2, described an inverse correlation between values of sclerostin and markers for bone formation (bone AP) and bone resorption (sCTX and TRAP 5b). Similar data have been reported in the general population[18] and in

patients immobilised after a vascular-cerebral accident[23], although in other situations, such as in patients with chronic renal insufficiency, no significant correlations have been observed[20,24,25]. In our study, although significant reductions in markers for bone formation (P1NP ad OC) were observed after treatment with GCC, these were related with neither Dkk-1 nor sclerostin.

The main limitations of this study were the reduced number of patients included, and the lack of follow up of these patients.

In conclusion, the effect of treatment with GCC on blood values of the Wnt pathway antagonists differs as a function of the antagonist being evaluated. While levels of Dkk-1 diminished at the start of treatment, the values of sclerostin showed no significant changes. All this suggests the necessity of carrying out prospective studies including a greater number of patients with better follow up to analyse the effect of the GCCs on the Wnt pathway antagonists.

Conflict of interest: The authors declare that there is no conflict of interest.

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Table 2. Correlation between values of sclerostin and values of Dkk-1, markers for bone remodelling and the other parameters analysed (Pearson r with and without adjusting for age[§])

	GCC (n=15)	Controls (n=20)
Age (years)	0.03/-	0.661*/-
BMI (kg/m^2)	0.254/0.267[§]	0.320/-0.036[§]
Markers of bone remodeling:		
PINP (ng/ml)	-0.295/-0.375[§]	-0.12/0.131[§]
OC (ng/ml)	-0.204/-0.212[§]	-0.593/0.134[§]
CTX (ng/ml)	-0.249/-0.360[§]	0.098/0.296[§]
Wnt pathway antagonists:		
Dkk-1	0.01/0.01[§]	-0.205/0.309[§]
BMD (g/cm^2):		
Lumbar	0.537*/0.651[§]*	-
Femoral neck	0.171/0.30[§]	-
Total femur	0.187/0.258[§]	-

GCC: glucocorticoids; BMI: body mass index; PINP: amino-terminal propeptide of pro-collagen type I; OC: osteocalcin; CTX: carboxy-terminal telopeptide of collagen type I; BMD: bone mineral density.
* $p<0.05$

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