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Circulating sclerostin and estradiol levels are associated with inadequate response to bisphosphonates in postmenopausal women with osteoporosis

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ABSTRACT

Introduction: The biological mechanisms associated with an inadequate response to treatment with bisphosphonates are not well known. This study investigates the association between circulating levels of sclerostin and estradiol with an inadequate clinical outcome to bisphosphonate therapy in women with postmenopausal osteoporosis.
Methods: This case-control study is based on 120 Spanish women with postmenopausal osteoporosis being treated with oral bisphosphonates. Patients were classified as adequate responders (ARs, $n = 66$, mean age 68.2 ± 8 years) without incident fractures during 5 years of treatment, or inadequate responders (IRs, $n = 54$, mean age 67 ± 9 years), with incident fractures between 1 and 5 years of treatment. Bone mineral density (DXA), structural analysis of the proximal femur and structural/fractal analysis of the distal radius were assessed. Sclerostin concentrations were measured by ELISA and 17β-estradiol levels by radioimmunoassay based on ultrasensitive methods.
Results: In the ARs group, sclerostin serum levels were significantly lower ($p = 0.02$) and estradiol concentrations significantly higher ($p = 0.023$) than in the IRs group. A logistic regression analysis was performed, including as independent variables in the original model femoral fracture load, 25 hydroxyvitamin D, previus history of fragility fracture, sclerostin and estradiol. Only previous history of fragility fracture (OR 14.04, 95% CI 2.38–82.79, $p = 0.004$) and sclerostin levels (OR 1.11, 95% CI 1.02–1.20, $p = 0.011$), both adjusted by estradiol levels remained associated with IRs. Also, sclerostin concentrations were associated with the index of resistance to compression (IRC) in the fractal analysis of the distal radius, a parameter on bone microstructure.
Conclusions: Sclerostin and estradiol levels are associated with the response to bisphosphonate therapy in women with postmenopausal osteoporosis.

Abbreviations: AR, adequate responders; IR, inadequate responders.

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1. Introduction

Postmenopausal osteoporosis is characterized by an increase in bone turnover and by an imbalance of bone resorption by osteoclasts over bone formation by osteoblasts [1,2]. These processes result in bone loss, affecting both bone mineral density and microarchitecture. As a result, the risk of fragility fracture is increased [3].

Treatment with bisphosphonates is effective in reducing fracture risk [4]. However, in clinical practice some patients suffer fractures in spite of being on therapy and with good adherence; such cases have been termed inadequate responders (IRs) [5]. More recently, a position paper of the International Osteoporosis Foundation has proposed the term treatment failure with stricter criteria based on the occurrence of two fractures while on treatment or one fracture plus lack of variation in bone mineral density (BMD), biochemical markers or both [6].

Bisphosphonate therapy in postmenopausal osteoporosis induces a rapid remodelling inhibition, decreasing bone resorption and bone formation, driven by the coupling between them. Wnt (Wingless-type) signalling is involved in this coupling process [7]. The activation of the Wnt/beta-catenin canonical signalling pathway stimulates osteoblastogenesis and inhibits osteoclastogenesis, shifting the balance toward bone formation and greater bone density [8].

Sclerostin is a Wnt pathway inhibitor preferentially expressed by osteocytes [8,9]. Sclerostin is directed through osteocytic canaliculi to the bone surface and binds to co-receptor LRP5–LRP6 (low density lipoprotein receptor-related protein 5 and low density lipoprotein receptor-related protein 6) [10–12]. In consequence, sclerostin inhibits the Wnt pathway, leading to decreased osteoblastogenesis and bone formation [11–13]. Genetic changes in the gene encoding sclerostin (SOST), with consequences in downregulation or loss of gene function, induce phenotypes with high bone mass. This is the case of sclerosteosis or van Buchem's disease in humans [14–16]. Conversely, transgenic overexpression of the SOST gene results in pronounced osteopoenia and reduced bone formation [17]. Phase II and III clinical trials are underway to assess the anti-fracture potential of anti-sclerostin antibodies in postmenopausal osteoporosis [18–20].

Serum sclerostin levels are lower in premenopausal than in postmenopausal women, and the serum levels of estrogen and sclerostin are negatively correlated [21]. Moreover, in postmenopausal osteoporosis, the withdrawal of estrogen therapy is accompanied by an increase in sclerostin [22]. Several reports show that estrogens mediate sclerostin expression in bone [21–24].

A recent study by the authors in women with postmenopausal osteoporosis while on antiresorptive medication (alendronate, risedronate or raloxifene) reported three independent variables associated with fractures [25]. These variables were prevalent fracture, low values of femoral fracture load and low levels of 25 hydroxyvitamin D. In the present study, we use this well-characterised cohort to identify IRs with respect only to bisphosphonate treatment (alendronate and risedronate), examining the circulating levels of sclerostin and estradiol. We also investigate the association between sclerostin levels and bone measures of structural/fractal analysis and BMD. We hypothesise that the increased expression of sclerostin, accompanied by estrogen deficiency, can influence the response to bisphosphonate therapy in women with postmenopausal osteoporosis.

2. Subjects and methods

2.1. Study design

This is a case-control study of patients on treatment with oral bisphosphonates, comparing cases with fractures (inadequate responders) with control patients who did not suffer fractures during treatment (adequate responders).

We investigated 120 postmenopausal women, from a cohort described elsewhere, which constituted the basis of the present study [25]. Sixty-six patients were considered adequate responders (ARs, control group) and they were included on completion of five years of bisphosphonate treatment provided that no fractures had occurred during 5 years of therapy. Fifty-four patients on bisphosphonate therapy were considered inadequate responders (IRs, case group), having suffered a fracture before completing five years on therapy and after receiving this treatment for at least 12 months (patients on bisphosphonate therapy with fractures before completing one year of treatment were not included in the study). This definition is simplified from an earlier one [5], according to which twelve months is a conservative estimate for assuming bisphosphonates reach full effect and that five years is the upper limit for top-quality evidence about efficacy. The Fig. 1 shows a flow chart of the study participants selection process and reasons for exclusions.

Physicians specializing in bone diseases recruited, assessed and included the study participants on the basis of medical records. Patients were recruited during routine medical visits and evaluated only once. Patients with previous bisphosphonate therapy were identified and in all cases, the duration of bisphosphonate therapy was recorded by the physician. Patients were evaluated by a questionnaire of risk factors for osteoporosis and fracture, which included the duration of use of drugs with potential effects on bone (thiazides, statins, beta-blockers, oral glucocorticoids, inhaled glucocorticoids, prior contraceptives, hormone-replacement therapy and also bisphosphonates).

Good compliance (at least 80%) was ensured before inclusion by directly questioning participants. The patient self report included questions regarding the difficulty in taking pills and forgotten pills in the last month. The dose compliance percentage in a period of a month was assessed by the formula (pills taken monthly $\times$ 100)/prescribed pills monthly. In addition, the adherence to treatment was evaluated applying the Morisky–Green questionnaire [26].

Patients were included as IRs if they suffered a low-impact fracture. Fractures of the skull, face, cervical spine, fingers and toes were not included. Spinal fractures had to be certified by radiograph in all cases. For nonvertebral fractures, a hospital discharge or physician's report was considered valid evidence of fracture.

Both groups of patients presented osteoporosis, defined by T-score < -2.5 at any location. The drugs considered were alendronate and risedronate. Exclusion criteria were prolonged glucocorticoid use (more than three months at any time), prolonged immobilisation (unable to walk independently, for three months or more) and disorders or treatments with bone effect. Other reasons for exclusion were previous or concomitant treatment with anticonvulsants, heparin, intravenous bisphosphonates, calcitonin, tibolone, teriparatide, parathyroid hormone (PTH) 1–84, or strontium ranelate, estrogen, estrogen-progestin or raloxifene. Patients receiving calcium supplements and/or Vitamin D (up to 1000 IU a day) were not excluded. Also, patients included in this study were treatment naïve prior to the 5 year use of bisphosphonates.

The study was carried out in twelve tertiary hospitals in Spain. The Ethics Committee at each institution approved the study protocol and informed consent was obtained from all individual participants included in the study for inclusion. In all cases, a questionnaire of risk factors for osteoporosis and fracture, including