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Effects of estradiol, calcitriol and both treatments combined on bone histomorphometry in rats with chronic kidney disease and ovariectomy

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Abstract

Background: The aim of this experimental study was to analyze the histomorphometric changes observed when using different doses of estradiol, calcitriol and both treatments combined, in rats with both chronic kidney disease (CKD) and ovariectomy (OVX).
Methods: Six groups of rats with CKD+OVX were treated for 8 weeks with placebo, with different doses of 17β-estradiol (E_2), with calcitriol or with both treatments combined (E_2+calcitriol). Histomorphometric studies were carried out at the proximal tibia segment.
Results: All groups that received active treatments showed a trabecular bone volume similar to those of rats with normal ovarian function. Treatment with E_2 was effective, E_2-10 diminished osteoid and eroded surfaces, and E_2-30 was able to achieve a bone remodeling similar to that of the normal group. Calcitriol proved to have a positive effect on bone microarchitecture, achieving normal trabecular connectivity. The combined treatment with E_2-30+calcitriol was the most effective treatment as it was not only capable of achieving normal trabecular remodeling and connectivity, but also normal trabecular bone volume.
Conclusions: E_2 and calcitriol seem to have independent effects on cancellous bone turnover in rats with CKD+OVX. In rats with chronic kidney disease and ovariectomy, these two agents are able to produce additive effects on bone and offer additional advantages as opposed to the use of both drugs independently.

Keywords: CKD-BMD; Bone formation; Bone resorption; Estrogen deficiency; Vitamin D replacement

Introduction

CKD-bone mineral disorder (MBD) is one of the main complications of CKD, which includes $1,25(OH)_2D_3$ deficiency, phosphate retention, calcium disorders and abnormal parathyroid function [12,24]. Estrogen deficiency could also be an additional risk factor in the development of accelerated bone disease in women with CKD.

Calcitriol and estrogens play an important role in bone metabolism through independent but synergistic mechanisms of action [1,6]. Studies in rats have shown that calcitriol prevents the loss of cancellous and cortical bone induced by estrogen deficiency due to the suppression of bone resorption [5]. In addition, administration of calcitriol improves intestinal calcium absorption, increases both serum calcium levels and urinary calcium excretion and inhibits parathyroid hormone (PTH) secretion [9].

Estrogen has been considered an effective treatment for the prevention and management of osteoporosis in postmenopausal women with normal renal function [2,20,23]. However, in women with chronic kidney disease (CKD), in whom disturbances in the hypothalamic–pituitary–gonadal axis are frequent [8,11], little is known about the role that estrogen deficiency plays in the pathogenesis and progression of bone disease [8,29]. Furthermore, the dose of estradiol needed to obtain safe benefits in CKD women has not been well defined [15,25,28]. Previous reports have suggested that kidney disease may alter the pharmacokinetic of estrogens [7], thereby making it difficult to extrapolate estrogen dose from normal women to women with CKD.

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Previous studies have already demonstrated beneficial effects of the combined treatment of estradiol and calcitriol – administered once per day 5 days/week – on bone in nephrectomized and ovariectomized rats [21,22]. In this study, we administered the estrogen using subcutaneous pellets as they reproduce better the current administration of estrogen replacement in women [4]. Estrogen alone or combined with calcitriol was evaluated using bone histomorphometry.

Animals and methods

Animals and diet

Six-month-old female Sprague–Dawley rats with a mean body weight of 310±26 g were used. The animals were kept under a standard rodent chow containing 0.6% calcium, 0.6% phosphorus and 1500 IU/kg of calcitriol (Diet A04, Panlab SL, Barcelona, Spain). Water administration was ad libitum.

Treatments

17β-Estradiol (Innovative Research of America, Sarasota, FL, USA) was administered as a pellet at a dose of 0.2 or 0.6 mg/pellet. The pellet was implanted in the space between the skin and muscle at the lateral side of the neck, between the ear and the shoulder. The 17β-estradiol (E_2) was released continuously during 8 weeks. Placebo pellets (0.6 mg excipient/pellet) (Innovative Research of America, Sarasota, FL, USA) were administered when needed using the same procedure. Calcitriol [$1\alpha25(OH)_2D_3$] (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in ethanol and adjusted with corn oil to a final volume of 0.8 ml corn oil/kg BW/injection. The final dose of calcitriol was 10 ng/kg body weight (BW)/day. This treatment was administered intraperitoneally (ip) 5 days per week for 8 weeks. Placebo (0.8 ml corn oil/kg BW/day) was administered following the same procedure.

Experimental design

CKD was obtained surgically by using the modified technique by Ormrod and Miller [17] (equivalent to 7/8 nephrectomy). Surgical estrogen deprivation was obtained by performing bilateral ovariectomy (OVX) [27]. Both procedures were done in the same surgical intervention using 42 mg/kg of ip ketamine (Ketolar, Warner-Lambert Company, NJ, USA) and 0.16 mg/kg of medetomidine (Dontor, Orion Corporation, Espoo, Finland) as anesthetics.

One week after the surgery, a total of 44 animals with CKD+OVX were divided into six experimental groups (Fig. 1).

Group 1 (placebo, $n=7$) received the two vehicles used in the two groups of active treatments: Corn oil (0.8 ml/kg/day) was administered through ip injections and the placebo pellet as described. Group 2 (E_2-10, $n=8$) received the vehicle (corn oil), injected ip and a pellet containing 0.2 mg of E_2 (10 μg/kg/day). Group 3 (E_2-30, $n=6$) received the vehicle (corn oil), injected ip and a pellet containing 0.6 mg of E_2 (30 μg/kg/day). Group 4 (calcitriol, $n=8$) received ip calcitriol (10 ng/kg/day), and a placebo pellet was also implanted. Group 5 (E_2-10+calcitriol, $n=8$) received the combined treatment with E_2-10+calcitriol. Estradiol was implanted as a pellet (0.2 mg of E_2) and calcitriol was administered ip (10 ng/kg/day). Group 6 (E_2-30+calcitriol, $n=7$) received the combined treatment with E_2-30+calcitriol. Estradiol was implanted as a pellet (0.6 mg of E_2) and calcitriol was administered ip (10 ng/kg/day).

A group of animals with CKD (same procedure) and normal ovarian function was used as the CKD control group ($n=5$) (Group 7). Some of the histomorphometric results were also compared with results from a group of animals of the same age and weight without any kind of manipulation (no CKD, no ovariectomy) previously published [22]. All rats were sacrificed by exsanguination after 8 weeks of treatment. On the 10th, 9th and 2nd days prior to sacrifice, animals received 20 mg/kg/day vibravenose (doxicicline, Phizer, Farmamondo, Switzerland), administered ip for bone fluorescent labeling. All the protocols were approved by the Laboratory Animal Ethics Committee of Oviedo University.

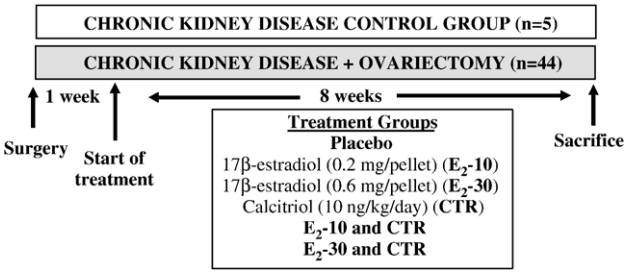

Fig. 1. Model of study and experimental design.

Analytical procedures

Serum markers

Blood samples were obtained after sacrifice, and serum urea, calcium and phosphorus were measured using a multichannel autoanalyser (Hitachi 717, Boehringer Mannheim, Berlin, Germany). The serum intact PTH (iPTH) was measured by IRMA (Rat PTH kit Inmunotopics, San Juan Capistrano, CA, USA). Uterus were also removed and weighed for using as marker of estrogen replacement.

Histomorphometry of the right proximal tibia

The one-third proximal segment of the right tibia was fixed in 70% ethanol, dehydrated, embedded in methyl-methacrylate and cut longitudinally in 5 μm sections using a Polycut S microtome (Reichert-Jung, Heidelberg, Germany). After deplastification, von Kossa–Ponceau stains were used. At least two non-consecutive sections per sample were examined.

A bone pathologist, blinded to the experimental code of the tissue, examined all samples. Static and structural parameters of bone formation and resorption were measured at a standardized site, below the growth plate in the secondary spongiosa, using a Leica DMRXA2 (Leica Microsystems, Wetzlar, Germany) optical microscope coupled to a digital video camera (Leica Microsystems Mod. Dc-100, Wetzlar, Germany) connected to an image analysis system (with appropriate image analysis software, Leica QWIN standard v.2.3, Leica Microsystems, Wetzlar, Germany). Parameters were reported according to the recommendations of Parfitt et al. [18], including trabecular bone volume (BV/TV, %); trabecular bone surface (BS/TV, mm^2/mm^3); osteoid surface (OS/BS, %); osteoblast surface (Ob.S/BS, %); total eroded surface (ES/BS, %); and osteoclast surface (Oc.S/BS, %).

Statistical analysis

Statistical analysis was performed using the Mann–Whitney test as a non-parametric test on the SPSS 8.0 software for Windows (SPSS Inc., Chicago, IL, USA). The results are expressed as a mean±standard deviation. Differences were considered significant when $p<0.05$.

Results

Serum markers

The analysis of the biochemical parameters at the end of the study is shown in Table 1.

Animals treated with calcitriol either alone or combined with estradiol (E_2-10+calcitriol and E_2-30+calcitriol) showed serum calcium and phosphorus levels significantly higher and serum PTH lower than those of the placebo group.

Animals treated with E_2-30 either alone or combined with calcitriol showed uterus weight values within the range observed in the CKD control group with normal ovaric function. Furthermore, an E_2 dose-dependent weight increase in the uterus was observed (E_2-10 vs. E_2-30 $p=0.029$; E_2-10+CTR vs. E_2-30+CTR $p=0.021$).