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Influence of estrogen therapy at conventional and high doses on the degree of mineralization of iliac bone tissue: a quantitative microradiographic analysis in postmenopausal women

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Abstract

The beneficial skeletal effects of menopausal estrogen replacement therapy (HRT) are well documented. The role of secondary mineralization of bone as a determinant of bone quality is now well established in postmenopausal women treated with bisphosphonates or SERMs. The aim of present study was to investigate the effect of conventional and high doses of estrogen on the main parameters reflecting the degree of mineralization of bone (DMB). Bone biopsies were obtained from 20 women with osteopenia or osteoporosis before and after 24 months (18 to 38 months) of conventional HRT, and from 19 women who had received high doses of estradiol (implant 100 mg every 3–6 months for 1.5–20 years). DMB parameters (mean DMB, DMB Freq. Max. and Heterogeneity Index of the individual distributions of DMB) were measured using quantitative microradiography in cortical, cancellous, and total bone and expressed as g mineral/cm^3 bone. Values obtained in women before HRT were lower than those reported in pre- and postmenopausal control women. After conventional HRT, there was an increase in mean DMB (total bone) of 4.4 ± 1.9% (mean ± SEM) versus pre-treatment values (4.1 ± 2.1% in cortical bone, 4.5 ± 2.3% in cancellous bone); these differences did not reach statistical significance ($P = 0.055$). Results were similar for DMB Freq. Max. but Heterogeneity Index was not significantly changed. After high dose estradiol therapy, mean DMB (total bone) was 6.9 ± 1.9% higher than in untreated women (8.6 ± 2.1% in cortical bone, 6.5 ± 2.1% in cancellous bone); this difference was statistically significant ($P \leq 0.03$). Results were similar for DMB Freq. Max. but once again Heterogeneity Index was not significantly modified. The increases in mean DMB were due to a shift of the curves towards high DMB with a decrease of the low DMB values, as confirmed by the absence of changes in the Heterogeneity Index. Estrogen therapy is associated with an increased degree of mineralization of bone induced by a prolongation of secondary mineralization, similar to that observed with other antiresorptive agents. However, this increase was about two-fold lower than that observed after alendronate therapy (10 mg/day/3 years) in postmenopausal osteoporotic women.

Keywords: Estrogen therapy; Degree of mineralization of bone; Heterogeneity of the mineralization; Quantitative microradiography; Postmenopausal women

Introduction

It is well established that estrogen has multifunctional roles influencing growth, differentiation, and metabolism in many tissues. It is an important factor in the maintenance of bone health, estrogen deficiency at the time of menopause being associated with bone loss. The beneficial skeletal effects of menopausal estrogen replacement therapy (HRT) are well documented [1–4]. Administration of estrogen at or after the menopause prevents bone loss and reduces fracture rate in the spine, hip and radius, effects which are believed to be mediated predominantly by inhibition of osteoclastic bone resorption. HRT preserves bone mass at both cortical and cancellous sites [5,6]. Histomorphometric analysis of

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cancellous bone in postmenopausal women has demonstrated that the beneficial effects of estrogen are predominantly due to suppression of bone turnover [7–10]. It has been recently suggested that estrogen may exert its antiresorptive effect on bone, at least in part, by stimulating estrogen receptors and osteoprotegerin expression in osteoblasts [11]. In women treated with conventional formulations of HRT, the effect on remodeling balance appears to be small and mainly due to decrease in the size of resorption cavities [10,12]. However, it has been recently demonstrated that long-term administration of high doses of estrogen produces anabolic effects in cancellous bone due to an increase in osteoblast activity [13,14].

High doses of estrogen have been shown to exert anabolic skeletal effects in postmenopausal women [13]. The first histomorphometric evidence in postmenopausal women of a dose-dependent estrogen-induced suppression of bone turnover in iliac crest cortical bone has recently been reported [5]. Significantly increased bone mineral density values have been reported in women treated long term with high-dose estrogen implant therapy [15]. Histomorphometric studies of cancellous bone have demonstrated the functional basis of this effect to be increased bone formation at the cellular level leading to increases in mean wall width and trabecular width [13,14]. In cortical bone, a tendency to an augmentation of wall width has also been reported [5].

The main determinants of bone quality are bone size and geometry, bone mass and density, bone microarchitecture and degree of mineralization at tissue level. The role of the degree of mineralization of bone (DMB), which reflects bone remodeling activity and the duration of secondary mineralization of bone, in the preservation of bone quality in treated postmenopausal osteoporotic (PMOP) patients is now well established [16–20]. In PMOP patients treated with bisphosphonates [21] or raloxifene [22], the gain of bone mineral density (BMD), measured by DXA at the lumbar spine, was almost exclusively explained by an increase of DMB although refilling of resorption cavities may also contribute.

Since DMB has not yet been quantified at tissue level after estrogen therapy and to better understand the mechanism of action of estrogen on mineralization, the present study investigated, in postmenopausal women, the effects of both conventional and high doses estrogen therapy on the mineralization of bone tissue.

Materials and methods

Patients and bone samples

Transiliac bone biopsies were obtained first from 20 postmenopausal women with osteopenia or osteoporosis, aged 54–71 years (mean age ± SD: 59.7 ± 7.1 years), before and after 18 to 38 months (mean: 23.5) of conventional HRT (paired biopsies, pre- and post-HRT groups, respectively), and second from 19 women aged 52–67 years (mean age ± SD: 56.2 ± 7.0 years) who had received long-term, high doses of estrogen implant therapy [100 mg every 3–6 months for 1.5–20 years (mean ± SD; 13.3 ± 5.4 years), high-dose estrogen group], following total abdominal hysterectomy and bilateral salpingo-oophorectomy for non-malignant disease [10,13]. These women formed part of two studies in which bone histomorphometry was reported previously [5,10,13]. Women receiving conventional HRT were treated for 2 years with a variety of oral or transdermal formulations (Prempak C or Premarin containing conjugated equine estrogens 0.625 mg/day; Trisequens containing estradiol 2 mg/day; Estraderm or Estracombi containing estradiol 50 μg/24 h). Women in the high-dose estrogen study had received estradiol implants, 100 mg, approximately 3–6 monthly on demand, although in some women the dose had been reduced to 50 mg every 6 months.

None of these women had a past or present history of illness associated with bone disease and none had taken drugs known to affect bone or mineral metabolism. Informed written consent was obtained from all patients and the studies were approved by the Local Research Ethics Committees.

Transiliac crest bone biopsies were obtained under local anaesthetic using a 7.5-mm internal diameter modified Bordier trephine. Samples were embedded in LR White medium resin (London Resin Co.). Thick sections (about 150 μm) were cut from bone samples using a precision diamond wire saw (Well, Escil, Chassieu, France), progressively ground to a thickness of 100 ± 1 μm, and polished with a diamond paste (1 μm). The thickness of the section was measured with an accuracy of 1 μm using a precision micrometer (Compac, Geneva, Switzerland). After cleaning with ultrasound, bone sections were then microradiographed [23].

Quantitative microradiography

Contact microradiography of 100 ± 1 μm-thick iliac bone sections was performed using an X-ray diffraction unit PW 1830/40 equipped with a diffraction tube PW 2273/20 (Philips, Limeuil Brévannes, France). The nickel-filtered copper Kα radiation was used under 25 kV and 25 mA. A Geola high-resolution film (VRP-M green sensitive) was exposed for 20 min (Slavich International Wholesale Office, Vilnius, Lithuania). For quantitative evaluation of the X-ray absorption by the bone section, a reference system composed of aluminum was exposed on each microradiograph [23,24]. The DMB was quantified using a new combined contact microradiography microdensitometry computerized method described briefly as follows [18,23,24]. A custom-developed software was used for the automatic analysis of gray levels of microradiographs with Visiolab 1000® (Biocom, France), a real color image processing workstation operating under Microsoft Windows®. The image of the