Bone 51 (2012) 714–719

Contents lists available at SciVerse ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Early effects of zoledronic acid and teriparatide on bone microarchitecture, remodeling and collagen crosslinks: Comparison between iliac crest and lumbar vertebra in ewes

N.R. Portero-Muzy [a,*], P.M. Chavassieux [a], M.L. Bouxsein [b], E. Gineyts [a], P. Garnero [a], R.D. Chapurlat [a]

[a] INSERM UMR 1033 et Université de Lyon, Lyon, France
[b] Orthopedic Biomechanics Laboratory, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA

ARTICLE INFO

Article history:
Received 7 March 2012
Revised 29 June 2012
Accepted 2 July 2012
Available online 14 July 2012

Edited by: Rene Rizzoli

Keywords:
Iliac crest
Vertebra
Ewe
Zoledronic acid
Teriparatide

ABSTRACT

Iliac crest bone biopsies are used to assess the mechanism of action of drug treatments, yet there are little data comparing this site to sites prone to fracture. The purpose of this study was to compare the delay and the amplitude of responses to treatment in two different bone sites. The short-term effects of zoledronic acid and teriparatide on microarchitecture, collagen crosslinks and bone remodeling were evaluated in iliac crest and lumbar vertebrae. Aged ewes (n = 8/gr) received either vehicle (CTRL) or a single injection of zoledronic acid (ZOL, 10 mg) or daily injections of teriparatide (TPTD, 20 μg/d) for 3 months. Blood samples were collected monthly for assessing bone turnover markers. At the end of the study, a transiliac bone biopsy (IC) and L1 lumbar vertebrae (LV1) were collected to assess bone microarchitecture; pyridinoline (PYD), deoxypyridinoline (DPD), pentosidine (PEN) content, static and dynamic parameters of bone remodeling. In CTRL, Tb-BV/TV was significantly higher in LV1 than IC ($p<0.0001$). This was associated with a trend of higher Tb.N, Tb.Th, DA, an inferior Conn.D and a lower bone turnover as shown by the decreases of osteoid parameters, MS/BS, Ac.f in LV1 when compared to IC. In addition, the ratio PYD/DPD was 4 times higher in LV1 than IC. After 3 months, significant decreases of sALP ($p<0.001$) and sCTX ($p<0.001$) were observed in the ZOL-group whereas in TPTD-group, after transient increases, they returned to baseline values. When compared to their respective CTRL, ZOL induced significant increases in Tb.BV/TV, Conn.D, Tb.N and Tb.Sp, in IC but not in LV1. Regardless of the site, ZOL markedly depressed the bone turnover: The static parameters of bone formation significantly decreased and the diminution of MS/BS, BFR/BS and Ac.f varied from −94 to −98% vs CTRL ($p<0.01$ to 0.001). It was associated with a diminution of the DPD content and the PYD/DPD ratio mainly in IC cortices. In contrast, after 3 months, TPTD did not modify the 3D structure and microarchitecture in IC and LV1, except a trend of higher Conn.D in IC, compared to IC-CTRL. TPTD treatment induced a significant increase in cortical porosity in LV1 ($p<0.05$) when compared to LV1-CTRL. Static parameters of bone formation and resorption were augmented in both sites, significantly only in LV1 ($p<0.05$) with a trend of increases in MS/BS and BFR/BS, compared to LV1-CTRL. In conclusion, in adult ewes, the bone mass, microarchitecture, remodeling and collagen crosslink content differ according to the bone site (iliac crest and vertebra). Furthermore, after 3 months, the responses to ZOL and TPTD were of different magnitude and delay between the two bone sites. The distinction of bone sites to study the early effects of anti-osteoporotic therapies appears meaningful in order to approach their site-specific anti-fracture efficacy.

Introduction

The effects of the anti-osteoporotic agents at the bone tissue level are often evaluated using transiliac bone biopsies. The iliac crest is the standard site for bone biopsy in humans, which is easily accessible, under local anesthesia [1]. Prior studies have reported strong correlations between trabecular bone volume at the iliac crest and lumbar vertebral body [2–4]. However, trabecular architecture is complex and several studies have demonstrated marked heterogeneity among skeletal sites [5,6]. Though less well studied, it is likely that other determinants of bone strength, including the composition of bone matrix and extent of microdamage, vary by skeletal site. Thus, we can hypothesize that the response of each of these determinants of bone strength to therapeutic interventions may also vary according to the skeletal site.

Zoledronic acid and teriparatide are two anti-osteoporotic agents with widely differing mechanisms of action. Zoledronic acid is a potent anti-resorptive agent whereas teriparatide increases both bone formation and resorption, with net anabolic actions. The long-term

* Corresponding author at: Faculté de Médecine Lyon-Est, Domaine Laënnec (La Buire), INSERM UMR 1033, Rue Guillaume Paradin, 69372 Lyon Cedex 08, France. Fax: +33 4 78 77 86 63.
E-mail address: Nathalie.Portero-Muzy@univ-lyon1.fr (N.R. Portero-Muzy).

8756-3282/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2012.07.004

effects of these two agents on bone tissue level properties have been described at the standard site of the bone biopsy i.e., the iliac crest [7–10]. However, the timing and the magnitude of responses to osteoporosis treatments may differ by skeletal site [11–15]. Analysis of the determinants of bone strength and their response to treatment at different skeletal sites may help to explain the variation in treatment efficacy for vertebral and non-vertebral fractures but also for cortical and non-cortical bones [12–16].

This study was conducted in aged ewes, because it is an animal model having a bone remodeling similar to postmenopausal osteoporosis women, previously used in bone histomorphometry studies [17–24]. The purpose of this present study was to assess the early changes in the determinants of bone strength at two different bone sites, the iliac crest and lumbar vertebrae, following anti-resorptive or anabolic therapeutic intervention in aged ewes. We tested the hypothesis that after 3 months of treatment, early changes in bone remodeling and several determinants of bone strength might differ across the different skeletal sites. The responses to anti-osteoporotic treatments might be of different magnitude and delay between the two bone sites, the iliac crest a standard site for bone biopsy and the lumbar vertebra a site prone to fracture. This would be relevant to approach the anti-fracture efficacy of different treatment regimes across different skeletal sites.

Material and methods

Animals

Twenty-four old ewes, all retired breeders, were randomly assigned to 3 groups based on minimizing group differences in age, weight, number of offspring and duration since the last lactation. The ewes were individually housed in an enclosed barn. They were kept under a constant photoperiod (16 h of light per day) for 2 months before the beginning and for the duration of the experiment, in order to reduce seasonal variations of bone remodeling. The animals were assigned to one of three groups ($n=8$/group) and received either vehicle (CTRL; mean age: 8.4 ± 0.4 yrs), a single injection of 10 mg of zoledronic acid (ZOL; mean age: 8.1 ± 0.4 yrs), or a daily subcutaneous injection of 20 μg of teriparatide (TPTD; mean age: 8.1 ± 0.4 yrs). All animals received elemental calcium (4.5 g/day) to normalize their dietary intake. After 3 months of treatment, the animals were euthanized. Prior to euthanasia, the animals received a double fluorochrome labeling according to the following schedule: 15 mg/kg/day of calcein (Calcein Disodium Salt, Fluka) for 2 days – 10 days off – then 4 days on, with euthanasia 2 to 5 days after the end of the second labeling period. The research protocol and animal procedures were approved by the ad-hoc committee from Institut National de Recherche Agronomique (INRA).

Serum biochemistry

Fasting morning blood samples were drawn every month and used to measure: serum bone specific alkaline phosphatase (sALP) by immunoradiometric assay [25] and serum crosslinked type I collagen C-terminal telopeptide (sCTX) by a two-site immunochemiluminometric assay using type 1 collagen monoclonal antibodies performed by an automatic analyzer (Elecsys, Roche Diagnostics, Mannheim, Germany) [26].

Bone specimens

At the end of the study, the left iliac crest (IC) and the first lumbar vertebrae (LV1) were harvested and immersed in 70% ethanol. Four bone biopsies were collected: two from the standard site of the iliac crest and two others from the central part of the vertebral body, with a low speed motorized 7.5 mm inner diameter trephine. IC and LV1 biopsies were approximately 1.5 and 3.5 cm long, respectively. The biopsies were kept in 70% ethanol.

Trabecular bone microarchitecture

The trabecular bone microarchitecture of IC and LV1 biopsies was evaluated in before embedding using a high-resolution desktop micro-tomographic imaging system (μCT40, Scanco Medical AG, Bassersdorf, Switzerland). Trabecular bone cores were aligned in the supero-inferior direction and the geometric midpoint was determined. MicroCT scans were acquired for 1 cm, centered at the midpoint, using a 20 μm^3 isotropic voxel size, 70 kVP, 114 mAs, and 200 ms integration time. Images were subjected to Gaussian filtration and then trabecular bone was segmented from background using a fixed threshold (21% of maximum gray scale value). Trabecular morphometry was determined within a 5 mm diameter core (to exclude bone pieces in the periphery that resulted from the coring process) using direct, 3D techniques that do not rely on any prior assumptions about the underlying structure. In particular, we assessed trabecular bone volume fraction (Tb-BV/TV, %), trabecular thickness (Tb.Th, mm); trabecular number (Tb.N, /mm); trabecular separation (Tb.Sp, mm); connectivity density (Conn.D, $/mm^3$); the degree of anisotropy (DA, #) and the structure model index (SMI). The methods adhered to published guidelines for assessing bone microarchitecture [27].

Bone histomorphometry

After μCT analysis, the IC and LV1 biopsies were embedded in methylmethacrylate without prior decalcification, according to previously described procedures [28]. For each sample we cut 8 μm thick sections at 3 different levels separated by 200 μm with a microtome (Polycut E, Reichert-Jung, Germany) and stained sections with either Solochrome cyanin R or Goldner's trichrome. Additional unstained sections were used for dynamic histomorphometry. Bone histomorphometric parameters were measured on the whole trabecular bone area on IC sections and on a region of interest matched with μCT analysis on LV1 sections. The parameters of bone structure and connectivity (strut analysis after skeletonization) were measured with an automatic image analyzer (Bone, Explora Nova®, La Rochelle, France). The static parameters reflecting resorption and formation, and the dynamic parameters of bone formation and mineralization were measured using a semi-automatic image analyzer (Tablet'Measure, Explora Nova®, La Rochelle, France). Bone histomorphometric parameters are computed and described in accordance with recommendations of the ASBMR Histomorphometric Nomenclature Committee [29]. We assessed 2D structural parameters, including the cortical thickness (Ct.Th, μm) and porosity (Ct.Po, %) and the cancellous bone volume (BV/TV, %). The parameters of microarchitecture (trabecular thickness (Tb.Th, μm), number (Tb.N, /mm) and separation (Tb.Sp, μm)) were derived from area and perimeter measurements according to the Parfitt's formulae [30]. The bone resorption was assessed by the eroded surfaces (ES/BS, %) and osteoclast number per bone surface (Oc.N, /mm). The static bone formation was reflected by osteoid surfaces (OS/BS, %), volume (OV/BV, %) and thickness (O.Th, μm). All these parameters were measured on Goldner-stained sections. The mineral apposition rate (MAR, μm/d) and the ratio of mineralizing surface to bone surface (MS/BS, %) were analyzed on unstained sections under ultraviolet light. The mean wall thickness (MWT, μm) was measured on Solochrome cyanin R-stained sections, under polarized light. Bone formation rate (BFR/BS, $(\mu m^3/\mu m^2/d) = (MS/BS)\times MAR$), adjusted apposition rate (Aj.AR, $(\mu m/d) = ((BFR/BS)/(OS/BS))$ and activation frequency (Ac.f, $(/yr) = (BFR/BS)/MWT$) were calculated. Strut analysis parameters were analyzed [31]. In our analysis, only the number of nodes/tissue volume (N.Nd/TV, $/mm^2$) was retained.