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Effects of teriparatide on cortical histomorphometric variables in postmenopausal women with or without prior alendronate treatment

Yanfei L. Ma [a], Qing Q. Zeng [a], Alan Y. Chiang [a], David Burr [b], Jiliang Li [c], Harald Dobnig [d], Astrid Fahrleitner-Pammer [d], Dana Michalská [e], Fernando Marin [f], Imre Pavo [f], Jan J. Stepan [g,*]

[a] Lilly Research Laboratories, Indianapolis, IN, USA
[b] Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA
[c] Department of Biology, Indiana University Purdue University, Indianapolis, IN, USA
[d] Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Graz, Austria
[e] 3rd Dept. of Internal Medicine, Charles University Faculty of Medicine 1, Prague, Czech Republic
[f] Lilly Research Centre, Windlesham, United Kingdom
[g] Institute of Rheumatology, and Charles University Faculty of Medicine 1, Prague, Czech Republic

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ABSTRACT

Cortical bone, the dominant component of the human skeleton by volume, plays a key role in protecting bones from fracture. We analyzed the cortical bone effects of teriparatide treatment in postmenopausal women with osteoporosis who had previously received long-term alendronate (ALN) therapy or were treatment naïve (TN). Tetracycline-labeled paired iliac crest biopsies obtained from 29 ALN-pretreated and 16 TN women were evaluated for dynamic histomorphometric parameters of bone formation at the periosteal, endocortical and intracortical bone compartments, before and after 24 months of teriparatide treatment. At baseline, the frequency of specimens without any endocortical and periosteal tetracycline labeling, and the percentage of quiescent osteons, was higher in the ALN than the TN group. Endocortical and periosteal mineralizing surface (MS/BS%), periosteal bone formation rate (BFR/BS), mineral apposition rate (MAR) and the number of intracortical forming osteons were significantly lower in the ALN-pretreated patients than in the TN group. Following teriparatide treatment, the frequency of endocortical and periosteal unlabeled biopsies decreased; in the ALN-pretreated group the percentage of quiescent osteons decreased and, in contrast, forming and resorbing osteons were increased. Teriparatide treatment resulted in significant increases of MAR in the endocortical, and MS/BS% in the periosteal compartment in the ALN-pretreated group. Most indices of bone formation remained lower in the ALN-pretreated group compared with the TN group at study end. Endocortical wall width was increased in both ALN-pretreated and TN groups. Cortical porosity and cortical thickness were significantly increased in the ALN-pretreated group after teriparatide treatment. Our results suggest that 24 months of teriparatide treatment increases cortical bone formation and cortical turnover in patients who were either TN or had previous ALN therapy.

Introduction

The human skeleton consists of about 80% cortical bone and this plays a critical role in supporting the whole body and in protecting bone from fractures [1]. The cortical shell makes a large contribution to bending rigidity in the femoral neck and is thinner in patients with osteoporotic fractures at this site [2,3]. Age-related bone loss is also characterized by a progressive increase in intracortical porosity and thinning of cortical bone [4]; consequently, advanced age is particularly associated with increased risk of fracture in predominantly cortical sites such as the hip, pelvis, upper leg and clavicle. Indeed, it has been reported that 80% of all fractures and most fracture-related morbidity and mortality in old age are accounted for by non-vertebral fractures in predominantly cortical sites [5]. Therefore, in the treatment of osteoporosis a drug that has a positive effect on cortical bone tissue, would confer maximum benefit in terms of fracture risk reduction [6].

The widely used antiresorptive drugs suppress bone turnover and decrease remodeling space, resulting in preservation of bone mass and a reduction in porosity [7]. While most antiresorptive agents result in a reduction of vertebral fracture risk by about 50%, they are less effective in preventing non-vertebral fracture where the reduction in risk is 20% to 30% [8]. In contrast, in a pivotal study of recombinant human parathyroid hormone fragment (hrPTH 1-34, teriparatide), administered to

* Corresponding author at: Institute of Rheumatology, Faculty of Medicine, Charles University, Na Slupi 4, CZ12850 Prague, Czech Republic.
E-mail addresses: ma_linda@lilly.com (Y.L. Ma), zeng_qing_q@lilly.com (Q.Q. Zeng), chiang_alan_y@lilly.com (A.Y. Chiang), dburr@iupui.edu (D. Burr), jilili@iupui.edu (J. Li), harald.dobnig@hormoninstitut-dobnig.at (H. Dobnig), astrid.fahrleitner@medunigraz.at (A. Fahrleitner-Pammer), d.michalska@post.cz (D. Michalská), marin_fernando@lilly.com (F. Marin), pavo_imre@lilly.com (I. Pavo), endojs@seznam.cz (J.J. Stepan).

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high risk postmenopausal women with osteoporosis, there was a reduced relative risk of vertebral fractures by 65% and non-vertebral fractures by 53% after 19 months of treatment [9]. Intermittent daily treatment with synthetic or recombinant PTH stimulates new bone formation by enhancing osteoblast activity and number due to increased differentiation and cell survival [10–12]. Direct bone formation on quiescent bone surfaces is also induced. These changes increase bone mass and improve microarchitectural characteristics of trabecular and cortical bone [12–22], contributing to increased bone strength [23–29].

Several histomorphometric studies on paired biopsies have provided evidence of increased static and dynamic indices of bone formation at the cortical level following teriparatide treatment. This was evident in the endocortical and periosteal compartments [15–21] but, so far, data regarding intracortical effects are limited [19,21,30].

Transient reductions followed by a delayed increase of areal BMD at skeletal sites with predominantly cortical bone have been reported with teriparatide treatment in patients with osteoporosis who have had long-term previous exposure to antiresorptive therapies [31–35]. Until now, no definitive histomorphometric studies have been carried out to evaluate the potential impact of previous antiresorptive treatment on the anabolic effect of teriparatide on cortical bone. The aim of the present study was to investigate the effect of a full teriparatide treatment cycle (24 months) on cortical bone structure and porosity, and cortical remodeling in patients who had previously been subjected to long-term alendronate therapy or who were osteoporosis treatment-naïve (TN).

Patients and methods

Study design and patients

This was a post hoc analysis from a prospective, single-arm treatment study of 66 post-menopausal women from two centers: Department of Internal Medicine 3, Faculty of Medicine, Charles University, Prague, Czech Republic, and Department of Internal Medicine, Medical University, Graz, Austria. Patients were at least 55 years of age with a total hip or lumbar spine T-score ≤ -2.5; 28 were TN and 38 had previously been treated with alendronate sodium (ALN) (10 mg/day or 70 mg/week), calcium (1000 mg/day) and vitamin D (800 IU/day) for a mean duration of 63.6 months. Eligibility criteria for enrolment have been reported previously [36,37]. Patients self-administered an injection of teriparatide 20 μg/day subcutaneously for 24 months, and took calcium (1 g) and vitamin D (400–1200 IU) as daily supplements.

Of the 66 Caucasian postmenopausal women originally enrolled, 45 had paired biopsies that qualified for histomorphometric measurements at both baseline and after 24 months of teriparatide treatment. Of these patients, 16 were TN and 29 had been pretreated with ALN. All 45 evaluable biopsy samples for mineralization and dynamic parameters of bone formation that had been used in a previous cancellous analysis were also valid for the dynamic histomorphometric measurements performed in the present cortical bone analyses [37].

Prior to the study each patient signed informed consent to the treatment and investigation protocol, which was approved by the Institutional Review Board for Research Involving Human Patients at both centers. All study methods and procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki.

Histomorphometry

The transiliac crest biopsies were obtained by manual drill using a 7.5 mm trephine (Medical Innovations International, Inc., Rochester, MN, USA). Biopsies were obtained at baseline prior to and after 24 months of teriparatide treatment. All patients took 250 mg tetracycline hydrochloride orally every 6 h for 2 days, 14 and 4 days prior to biopsy under conditions described previously [18]. Biopsy samples were stored and transported in 70% ethanol to the central bone histomorphometry laboratory for preparation of histological sections (DB Burr, Indianapolis, IN, USA). For the current study one section with McNeal's tetrachrome stain and one unstained section, used in our previous cancellous bone analyses [37], were reused for bright field or fluorescence microscopic analyses. The mean wall width was measured on toluidine blue cement line specific stained sections, under bright field and polarized light. All 45 pairs of biopsies analyzed in the previous study were included in the current study, even though some specimens only exhibited the fluorochrome label on cancellous surfaces and not on endocortical or periosteal surfaces. If one unstained section did not show tetracycline fluorescence or exhibited single label (sL) only, a second section was examined and used for fluorochrome measurements if there was detectable label. The second section was examined in more than half of the biopsies for both endocortical and osteonal labeling measurements. Two sections were examined for all biopsies to select the one with most labeling activity (more labeling, or longer double-label) for the periosteal labeling measurement.

Histomorphometric analyses were performed semi-automatically by a direct tracing method using a Digitizing Image Analysis System consisting of an epifluorescent microscope and digitizing pad (Summagraphic, Fairfield, CT, USA) coupled to a computer with histomorphometry software (KSS Scientific Consultants, Magna, UT, USA) [19,38]. Measurements were carried out under $\times 100$ magnification on the entire periosteal and endocortical bone envelopes, with the exception of the intracortical resorbing osteon, where measurements were carried out under $\times 200$ magnification. The cortical dynamic and cortical porosity static assessments were performed by two readers who were blinded to group affiliation.

Mean cortical thickness and cortical volume were measured quantitatively with an image probe system on 3D CT images (EVS, now General Electric, London, Ontario, Canada). Porosity area was measured quantitatively on histology sections; only osteons with a diameter larger than 30 μm were included in the analyses. The number of bone-forming osteons was calculated as the sum of osteons with osteoblasts, osteoid or visible labeling, the number of bone-resorbing osteons was the sum of the osteons with osteoclasts, and the number of quiescent osteons were those that exhibited no activity indicating formation or resorption [26]. Single-label (sL) length, double-label (dL) length and interlabel width, were measured at endocortical, periosteal and intracortical surfaces. Wall width was measured in the endocortical surface only. The mineralizing surface (MS%) was calculated as dL length plus half sL length (MS/BS% = [dL + 1/2sL] / bone surface [BS] × 100). Osteoblast, osteoclast and osteoid surfaces were measured on endocortical and intracortical surfaces and normalized to percent BS. For specimens without a sL or dL, a zero was assigned and their respective mineral apposition rates (MAR) and bone formation rates (BFR)/BS were accordingly zero (MAR1) [39,40]. We also applied two additional methods of calculating MAR for those specimens without dL regardless of sL status; either an imputed value of 0.3 μm/day was assigned (MAR2), or the sample was treated as a missing value (MAR3) and BFR/BS was calculated accordingly (Table 1) [40]. All cortical data presented in this study were from both cortices, and measured and derived variables were expressed in accordance with the histomorphometric criteria recommended by the subcommittee on bone histomorphometry of the American Society for Bone and Mineral Research [41].

Table 1
Different calculations used for mineral apposition rate (MAR) and bone formation rate (BFR)/bone surface (BS) analyses.

Index	dL[a]	MS	MAR	BFR/BS
MAR 1	0	1/2 sL	0	0
MAR 2	0	1/2 sL	0.3 μm/day	Calculation
MAR 3	0	1/2 sL	Missing value	Missing value

dL = double label, MS = mineralizing surface, sL = single label.
[a] Only double label is considered regardless of presence or absence of single label.