Bone 48 (2011) 1154–1163

Contents lists available at ScienceDirect

Bone

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

Ibandronate does not reduce the anabolic effects of PTH in ovariectomized rat tibiae: A microarchitectural and mechanical study

Xiao Yang [a,1], Yong Hoow Chan [a,1], Padmalosini Muthukumaran [a], Shamal DasDe [b], Swee-Hin Teoh [c], Taeyong Lee [a,*]

[a] Division of Bioengineering, Block E1, #08-03, 9 Engineering Drive 1, National University of Singapore, 117576 Singapore
[b] Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University Hospital, Singapore
[c] Department of Mechanical Engineering, Faculty of Engineering, National University of Singapore, Singapore

ARTICLE INFO

Article history:
Received 23 November 2010
Revised 18 January 2011
Accepted 4 February 2011
Available online 18 February 2011

Edited by: R. Recker

Keywords:
Ibandronate
PTH
Additive effect
Bone morphology
Micro-CT

ABSTRACT

Osteoporosis remains a challenging problem. Understanding the regulation on osteoclast and osteoblast by drugs has been of great interest. Both anabolic and anti-resorptive drugs yield positive results in the treatment of osteoporosis. However, whether the concurrent administration of parathyroid hormone (1–34) and ibandronate may offer an advantage over monotherapy is still unknown. This study, therefore, attempts to compare the efficacy of two therapeutical approaches and to investigate the beneficial effects in concurrent therapy in a rat model using three-point bending, pQCT and μCT analysis. A total of 60 female Sprague–Dawley rats of age 10 to 12 weeks were divided into 5 groups (SHAM, OVX + VEH, OVX + PTH, OVX + IBAN, OVX + PTH + IBAN) and subjected to ovariectomy or sham surgery accordingly. Low-dose parathyroid hormone (PTH) and/or ibandronate or its vehicle were administered subcutaneously to the respective groups starting from 4th week post-surgery at weekly intervals. Three rats from each group were euthanized every 2 weeks and their tibiae were harvested. The tibiae were subjected to metaphyseal three-point bending, pQCT and μCT analysis. Serum biomarkers for both bone formation (P1NP) and resorption (CTX) were studied. A total of 11 indices showed a significant difference between SHAM and OVX + VEH groups, suggesting the successful establishment of osteoporosis in the rat model. Compared to the previous studies which showed impedance from bisphosphonates in combination therapy with PTH, our study revealed that ibandronate does not block the anabolic effects of PTH in ovariectomized rat tibiae. Maximum load, strength–strain indices and serum bone formation markers of OVX + PTH + IBAN group are significantly higher than both monotherapy groups. With the proper ratio of anabolic and anti-resorptive drugs, the effect could be more pronounced.

Introduction

Osteoporosis is a progressive debilitating disease that often leads to pain, nerve compression and even fracture caused by the reduction in bone strength and compromised stability [1–3]. After years of clinical studies in the treatment of osteoporosis, two distinct classes of drugs, namely the anabolic and anti-resorptive agents, proved to be effective and are now available on the market. Intermittent administration of parathyroid hormone (PTH) has been shown to have an anabolic effect on bone structural properties in animal models [4] and humans [5,6]. It increases bone strength primarily by stimulating bone formation. Alternatively, third generation nitrogen-containing bisphosphonates such as ibandronate and zoledronate have been successfully used to prevent fracture by suppressing bone resorption [7–10]. Ibandronate has been proven effective in inhibiting bone resorption at considerably lower doses than other bisphosphonates in both rats [11] and ovariohysterectomized dogs [12,13]. Following long-term treatment with ibandronate, bone mass, strength and architecture were maintained, or even improved [14].

Whether the concurrent use of bisphosphonate and PTH would produce an additive effect by reducing fracture risks is still controversial [5,15–19]. Black et al. have reported that a combined therapy of PTH (1–84) and alendronate has no synergistic effect on postmenopausal women, in terms of changes in bone mineral density (BMD) and mean percent changes in biomarkers of bone metabolism [5]. However, BMD measurement does not provide sufficient evaluation of the trabecular architecture [20], actual stiffness or the ability to withstand an applied force on bones [21]. Furthermore, it is becoming generally accepted that high BMD does not always relate to low fracture risk [22–25]. Additionally, a recent ovariectomized rodent study discovered that the effect of combined administration of alendronate and PTH (1–34) on bone strength is synergistic in the

* Corresponding author. Fax: +65 6872 3069
E-mail address: bielt@nus.edu.sg (T. Lee).
[1] These authors contributed equally.

8756-3282/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2011.02.007

lumbar vertebra and additive in the femur [18]. In order to ensure the efficacious use of pharmaceutical materials, it is essential to investigate the interaction and the net effect of parathyroid hormone with newly approved bisphosphonates. From another aspect, it provides a basic understanding on drug combinations for future consequential or alternative administration.

Therefore, we conducted a study to see whether there is a beneficial effect of the anabolic and anti-resorptive therapies in the ovariectomized rats. We hypothesized that the combination therapy with PTH (1–34) and ibandronate would offer an advantage over monotherapy, in terms of bone mineral density combined with micro-architectural changes [26] and eventually bone strength. The weekly low dosages of both PTH and Ibandronate adopted in this study were selected respectively from individual dosing tests and also served to circumvent the negative side effects present in higher doses[14,27,28]. To test this hypothesis, we compared the micro-architectural and mechanical changes of OVX rat tibiae in the following three treatment groups: PTH (1–34) alone, ibandronate alone and combined administration of PTH (1–34) and ibandronate.

Materials and methods

Overall study design

Sixty female Sprague–Dawley rats (Laboratory Animal Centre, National University of Singapore) aged 10 to 12 weeks were housed at 25 °C under a 12:12-hour light–dark cycle. They were fed a standard rodent diet (Harland, Model T.2018S) and water *ad libitum*. The animals were subjected to OVX or sham surgery 1 week after acclimatization at the animal holding unit. All animal experiments were conducted in accordance with the approved protocol from the Institutional Animal Care and Use Committee (IACUC) at the National University of Singapore.

The rats were divided into 5 groups (SHAM, OVX + VEH, OVX + PTH, OVX + IBAN and OVX + PTH + IBAN) with weekly subcutaneous administration of either saline vehicle, PTH, ibandronate or both drugs. The study was carried at 6 predetermined time points for SHAM (0, 4, 6, 8, 10 and 12 weeks) and OVX (2, 4, 6, 8, 10, 12 weeks) and 4 time points for treatment (6, 8, 10 and 12 weeks). There were 12 animals in each group, generating 24 samples (both tibiae) per group, with $n = 6$ samples per time point for each treatment (OVX + PTH, OVX + IBAN and OVX + PTH + IBAN) and $n = 4$ samples per time point each for OVX and sham control groups (OVX + VEH and SHAM). The animals did not exhibit signs of distress or illness from the surgery or drug treatments during the course of the study and none were excluded from the study.

Human parathyroid hormone (PTH 1–34, Sigma-Aldrich, Singapore) and ibandronate (Roche Diagnostics GmbH, Mannheim, Germany) were diluted with 0.9% saline. From 4th week post-surgery, PTH (1–34) (10 μg/kg body weight) [27], ibandronate (7 μg/kg body weight) [28] or its vehicle (0.9% saline) was administered subcutaneously once a week for 8 weeks to the respective groups.

Every 2 weeks, two animals from SHAM and OVX and three from each treatment groups were euthanized by carbon dioxide asphyxiation. Both right and left tibial bones were harvested, wrapped in 0.9% saline soaked guaze and stored at −20 °C until used for the experiments. From the study of osteoporotic bone fracture using rat models, the majority of fractures was observed at the metaphysis of long bones [21] where a rapid deterioration of trabecular bone occurs [29]. Thus, the trabecula-abundant metaphyseal tibia is a suitable region to determine the degree of osteoporosis by bending tests [21,30] and 3D micro-architectural analysis of trabecular bone [29,31] in ovariectomized (OVX) rat models.

Micro-computed tomography (μCT)

The metaphysis region (Fig. 1A) of the right proximal tibia was scanned *ex vivo* in an upright position with a source to object distance (SOD) of 41 mm and a source to image distance (SID) of 339 mm [32] using a SMX-100CT μCT scanner (Shimadzu, Kyoto, Japan). A 3.29-mm-thick volume of interest (VOI, 200 slices) was obtained starting from 1 mm distal to the proximal growth plate [26]. The resultant grayscale images obtained had an isotropic voxel size of 16.47 μm from cone-beam reconstruction (46 kV, 49 μA, scaling coefficient of 50 and averaged 3 times).

A semi-automated contouring method was employed to select trabecular from cortical bone. The grayscale images were segmented using a global threshold of 15.0% of the maximal grayscale value [33] using the CT Analyzer software (Skyscan, Phil Salmon). Three-dimensional analyses [32] was used to assess bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), structural model index (SMI) and trabecular porosity (Tb.Po) for the same VOI.

Peripheral quantitative computed tomography (pQCT)

The pQCT scans were carried out with a pixel size of 0.1 mm and a slice thickness of 0.5 mm using a StraTEC's XCT machine (Research SA +, StraTEC Medizintechnik, GmbH, Pforzheim, Germany). A scout view was performed prior to the actual scan to enable exact positioning of the bone specimens. The region of interest selected was similar to that used for μCT where the primary effect of osteoporosis was expected to be significant. Three types of volumetric bone mineral density (vBMD) were obtained, i.e., mean BMD, trabecular density (Tb.BMD) and cortical density (Ct.BMD). The thresholds used for separating soft tissue from bone and sub-cortical

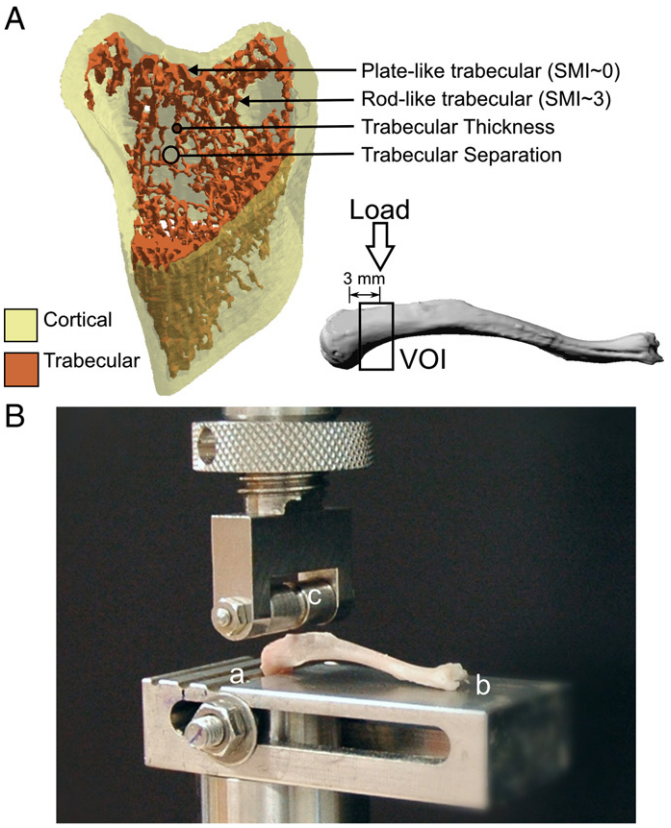

Fig. 1. The tibia specimen in the testing position. (A) μCT rendered image of rat tibia. (B) Details of the three-point bending test consisting of the aluminum base and the roller stamp.