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Technical Note

The use of micro-CT to evaluate cortical bone geometry and strength in nude rats: Correlation with mechanical testing, pQCT and DXA

Cedo M. Bagi [a,*], Nels Hanson [a], Catharine Andresen [a], Richard Pero [a], Roland Lariviere [a], Charles H. Turner [b], Andres Laib [c]

[a] *Comparative Physiology and Medicine, Safety Sciences, Pfizer Inc, Eastern Point Road 8274-1312, Groton, CT 06340, USA*
[b] *Depts. of Orthopaedic Surgery and Biomedical Research, Indiana University Medical Center, Indianapolis, IN 46202, USA*
[c] *SCANCO Medical AG, Bassersdorf, Switzerland*

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Abstract

In both clinical and experimental settings, access to quantitative methods enabling the objective evaluation of cortical bone mass, structure, geometry and strength are essential for the assessment of efficacy and safety of different treatments aimed to improve bone strength. The ability of non-invasive methodologies (DXA, pQCT and micro-CT) to assess and quantify cortical bone mass and geometry was tested in a nude rat model in which bone loss was induced by surgical castration. Treatment with a bone antiresorptive (alendronate) or a bone forming (PTH) drug was used to: (A) validate the nude rat model in terms of bone metabolism, (B) test the ability of each technology to detect change in cortical bone geometry and (C) correlate cortical bone geometry with bone strength data obtained by 3-point bending method.

Our observations regarding effect of castration and treatment with PTH and alendronate on cortical bone parameters in nude rats is in general agreement with previously published data obtained in immunocompetent male rats under similar experimental conditions. Data presented here support the hypothesis that nude rats have similar bone physiology and response to known bone therapies to that observed in normal rats and therefore could be effectively used to predict skeletal response in humans.

All three technologies deployed in this study (DXA, pQCT and micro-CT) proved useful in describing cancellous and/or cortical bone parameters and positive correlations were demonstrated between data obtained by different methods. The cross-sectional area of a bone structure is crucial for resisting loads in bending or torsion and is described as "areal moment of inertia" for bending, and as "polar moment of inertia" in torsion. Novel, three-dimensional micro-CT methodology used in this study to assess geometry of cortical bone provides data that accurately describes cortical bone geometry and parallels cortical bone strength results obtained by the 3-point bending method. Our micro-CT data meet the criteria of providing quick, reproducible and accurate answers regarding cortical bone geometry as a predictor of cortical bone strength.

Keywords: Cortical bone; Micro-CT; pQCT; DXA; Mechanical testing; Nude rats; PTH; Alendronate

Introduction

The skeleton is a mechanically optimized biological system whose composition and organization reflect the functional demands made upon it. The physical characteristics of bone including the morphology, dimensions and distribution of its composite apatite crystals affect its mechanical properties [1–6]. The cortical compartment makes up about 85% of the skeleton and is mainly responsible for mechanical strength and stiffness, while the cancellous compartment has both a mechanical and a metabolic role serving as a reservoir for calcium and phosphorus. Apatite crystals are one of the major constituents of bone tissues in vertebrates. Their presence accounts for approximately 65% of bone weight and provides most of the strength and stiffness of bone and allows for use of radiological technologies to assess bone mass and structure [7,8].

In order for a scientist or physician to measure bone strength and chose treatment modalities, an objective quantitative method for assessment of bone structure is highly desirable. Cortical bone geometry and strength have traditionally been

* Corresponding author. Fax: +1 860 715 3577.
E-mail address: cedo.bagi@pfizer.com (C.M. Bagi).

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evaluated ex vivo by using a combination of histomorphometry and mechanical testing. Static histomorphometry of the cortical bone takes significant time to complete, involves labor-intensive procedures, requires specialized equipment and training and is subject to inconsistencies due to the multiple techniques involved [9–12]. Development of imaging technologies such as dual energy X-ray absorptiometry (DXA), peripheral Quantitative Computed Tomography (pQCT) and micro-Computed Tomography (micro-CT) has markedly improved our ability to assess structural parameters of cancellous and cortical bone, and therefore to accurately diagnose skeletal disorders and monitor progression of the disease and effect of therapeutic interventions [13–22].

In the current study, we sought to determine the manner in which the diaphysis adapts structurally and biologically to treatment with a bone forming (PTH) or a bone antiresorptive (alendronate) agent in intact and castrated male immuno-deficient rats. We compared DXA, pQCT and micro-CT data with bone strength results obtained by the 3-point bending method. As far as we know, this is the first attempt to estimate strength of the cortical bone by assessing cortical bone geometry with micro-CT and to directly compare bone geometry measurement by various imaging modalities with actual bone strength obtained by mechanical testing.

Material and methods

Animals

Nude (Crl:NIH-rnu) male rats were purchased from Charles River (Wilmington MA). Rats arrived at 10 weeks of age and were maintained according to the NIH standards established in the "Guidelines for the Care and use of Laboratory Animals". The Internal Animal Care and Use Committee (IACUC) approved all experimental protocols. The rats were pair housed in polycarbonate micro-isolator cages lined with autoclaved bedding. Autoclaved reverse osmosis (RO) water and autoclaved standard rat chow were provided ad libitum. Bone loss was induced by surgical castration 11 days before the start of dosing and under isoflurane anesthesia following aseptic procedure guidelines. Body weights were recorded weekly throughout the course of the studies. Animals were maintained for 6 weeks and euthanized by CO_2 inhalation at the end of the experiment. The animals were randomized to study groups by body weight. There were six experimental groups: Intact controls, Intact + PTH, Intact + Alendronate, Castrate controls, Castrate + PTH and Castrate + Alendronate.

Human PTH (1–34)

Human PTH (1–34) (Bachem, King of Prussia, PA) was formulated for subcutaneous dosing in 0.001 N HCl with 5% heat inactivated rat serum and delivered at 80 μg/kg QID, 5× per week.

Alendronate

Alendronate (Pfizer Inc., Groton CT) was formulated for subcutaneous dosing in sterile saline and delivered at 0.28 μg/kg QID, 5× per week.

Blood collection

Blood was collected by retro-orbital bleed for hematology and serum chemistry and biomarker measurements. Animals were lightly anesthetized during the bleed procedure with CO_2/O_2. Hematology parameters were analyzed using an Advia 120 (Bayer, Germantown NY). Chemistry endpoints were analyzed using a Hitachi 917 auto-analyzer (Roche, Indianapolis, IN). Terminal blood was taken under CO_2 anesthesia by cardiac puncture. Serum endpoints evaluated included: testosterone (Alpco, Windham NH), osteocalcin (Alpco, Windham NH) and rat PTH (Immutopics, San Clemente CA).

Other procedures

Fluorochrome bone markers were injected at baseline and just prior to termination of the study. Calcein (Sigma, St Louis, MO) was prepared at 10 mg/ml in 2% sodium bicarbonate/saline and injected at 10 mg/kg. Demeclocycline (Sigma) was prepared at 15 mg/ml in sterile saline and injected at 15 mg/kg. Both fluorochromes were delivered by giving half the dose volume as intra-peritoneal and half as subcutaneous injection.

Necropsy

Animals were euthanized by CO_2 inhalation. The femurs and tibias were dissected, snap-frozen in liquid N_2 and stored at −80°C for radiological and mechanical testing or in 10% formalin for histological analyses.

Imaging

At necropsy, both femurs were collected from each rat. Right femurs were flash-frozen and stored at −80°C prior to submission for bone assessment by DXA, pQCT and mechanical testing. Left femurs were gently cleaned of soft tissue, and stored in 10% buffered formalin. Prior to submission for micro-CT analysis, a 5 mm section of the femoral diaphyses (midshaft) was isolated using a "Dremal" saw system (Racine, Wisconsin) (Fig. 1). Following micro-CT scans, the midshaft samples were returned to 10% buffered formalin and used for undecalcified bone histology.

Micro-CT imaging

Details regarding the MicroCT-40 computed tomography system (Scanco Medical, Bassersdorf, Switzerland) and analysis software used in this study have been described previously [23]. Briefly, the unit consists of an X-ray source

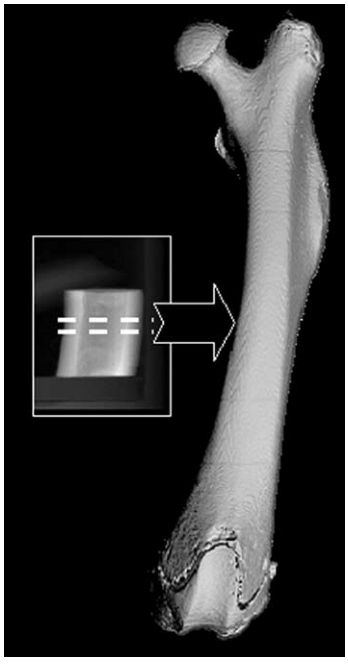

Fig. 1. The image of the entire femur was taken using micro-CT. Arrow indicates femoral diaphysis (midshaft) that was dissected and used for micro-CT analysis (rectangle) of the cortical bone parameters. The doted lines indicate cortical bone used for 2D and 3D micro-CT analyses.