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Analysis of cortical bone porosity using synchrotron radiation microtomography to evaluate the effects of chemotherapy

R. Alessio [a,*], L.P. Nogueira [b], C. Salata [c], A. Mantuano [b], A.P. Almeida [a], D. Braz [a], C.E. de Almeida [c], G. Tromba [d], R.C. Barroso [b]

[a] Nuclear Instrumentation Laboratory, COPPE/UFRJ, Rio de Janeiro, Brazil
[b] Lab of Appl Phys to Biol and Envior Sci/Physics Institute, State University Rio de Janeiro, Rio de Janeiro, Brazil
[c] Radiological Sciences Laboratory, State University of Rio de Janeiro, Rio de Janeiro, Brazil
[d] Sincrotrone Trieste SCpA, Trieste, Italy

HIGHLIGHTS

- Changes in cortical bone porosity due to chemotherapeutic drugs.
- 3D morphometric parameters with synchrotron radiation microtomography.
- Chemotherapy induced bone loss in rats.

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ABSTRACT

Microporosities play important biologic and mechanical roles on health. One of the side effects caused by some chemotherapy drugs is the induction of amenorrhea, temporary or not, in premenopausal women, with a consequent decrease in estrogen production, which can lead to cortical bone changes. In the present work, the femur diaphysis of rats treated with chemotherapy drugs were evaluated by 3D morphometric parameters using synchrotron radiation microtomography. Control animals were also evaluated for comparison. The 3D tomographic images were obtained at the SYRMEP (SYnchrotron Radiation for MEdical Physics) beamline at the ELETTRA Synchrotron Laboratory in Trieste, Italy. Results showed significant differences in morphometric parameters measured from the 3D images of femur diaphysis of rats.

1. Introduction

Cortical bone is characterized by a complex and dynamic microstructure that cannot be fully defined with current 2D analytical methods (Cohen and Harris, 1958). Microstructural alterations have a significant impact on the material properties of cortical bone (Yeni and Norman, 2000; McCalden et al., 1993), and also affects localized fluid pressure gradients (Wang et al., 1999) and streaming potentials (Starkebaum et al., 1979).

Some chemotherapy agents can cause significant bone loss, in the trabecular and cortical bones (Datta and Schwartz, 2013). One of the side effects caused by chemotherapy drugs is the induction of amenorrhea, temporary or not, in premenopausal women, with a consequent decrease in estrogen production. It leads to bone changes, similar to those presented in osteoporosis (Hadji et al., 2012). The changes occurring in bone architecture due to decreased estrogen production, caused by chemotherapy, are similar to those caused by ovariectomy and the effects of age (Marcus et al., 2008). Understanding the progression of estrogen-deficient osteoporosis is necessary in order to reduce the risk of fractures, sickness, and death among women submitted to chemotherapy treatment. An important step to better comprehend bone alterations induced by this treatment is to characterize the changes that occur within the microporosity in cortical bone.

Numerous studies have reported the importance of porosity with respect to cortical bone strength and elasticity (Currey, 1988; Schaffler and Burr, 1988; Martin and Ishida, 1989). McCalden et al. (1993) reported that age-related increase in cortical porosity is the major contributor to the decrease in the mechanical bone properties, accounting for 76% of the loss of bone strength with age. Differences in the distribution of cortical porosity in the femoral neck, with increased porosity concentrated in the anterior region have been associated with increased fracture risk (Bell et al., 1999).

* Corresponding author. Fax: +55 212334 0699.
E-mail address: ritavita12@gmail.com (R. Alessio).

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In addition to the percentage of overall porosity, the dimensions of the individual canals and their spatial arrangement have also been implicated. Previous studies, utilizing microtomography, determined that the vascular porosity in the cortical tibia metaphysis of rat bone increases by 32% when assessed 6 weeks after ovariectomy. An increase in vascular canal diameter was also reported in this same region in ovariectomized rats causing a decrease in bone mechanical properties (Larriera et al., 2010).

Synchrotron radiation combined with microtomography (SR-μCT) has opened up new possibilities in 3D analysis of cortical microstructure (Dilmanian, 1992; Peyrin et al., 2000). The synchrotron radiation properties of natural collimation and extremely high light intensity, even after monochromatization, enable SR-μCT to reconstruct the highly resolved 3D image with a high signal-to-noise ratio, besides eliminating beam-hardening artifacts and, therefore, allowing a more precise image quantification for determining local cortical bone morphometrical parameters. Furthermore, 3D structural analysis will ultimately improve our understanding of cortical bone mechanics and the stimuli that regulate the remodeling process. Methodological limitations have presented the primary hurdle to 3D analysis of cortical bone. However, μCT has an effective means of visualizing and quantifying the 3D structure of cortical bone porosity at the level of the osteonal canals (Gruber et al., 2005; Matsumoto et al., 2006; Cooper et al., 2006; Larriera et al., 2010). The limitation of μCT to porosity has precluded a conventional analysis of remodeling based upon quantitative analysis of secondary osteons. Therefore, the 3D structure of cortical porosity at this level potentially provides a window into the 3D dynamic structure of this tissue.

The purpose of this study was to evaluate quantitative changes in the intracortical bone architecture of rats treated with a combination of chemotherapy drugs through morphometrical parameters, utilizing 3D computed microtomography.

2. Materials and methods

2.1. Sample preparation

Female Wistar rats ($n=10$), three months old, weighing approximately 200 g, were kept on a 12-h light/dark cycle with food and water provided *ad libitum*. Rats were obtained from the Laboratory of Radiological Sciences (LCR-UERJ, Rio de Janeiro, Brazil). The animals were divided into two groups, five animals each. The treated group received doses of docetaxel and cyclophosphamide drugs (G1) while the control group (G0) received no further treatment, being used as a parameter of normal bone structure. In the chemotherapy group, the multidrug was intraperitoneally administered to the animals. They were given four cycles, with an interval of seven days between them, each one of the animals received 50 mg/kg of cyclophosphamide and 12.5 mg/kg of docetaxel. The dose of each drug, in each cycle, was calculated to be equivalent to a chemotherapy dose per cycle in humans by the Human Equivalent Dose Calculation (FDA, 2012). The rats were sacrificed by direct heart injection of KCl at 150 days post-treatment (240 days of life), and femurs were excised, cleaned and let to air dry for at least 72 h. Ethics permission to utilize the animals for the research described in this paper was obtained from the Ethics Committee on Animal Research of the State University of Rio de Janeiro (Process CEA/010/2012).

2.2. Syrmep beamline

The experiments were performed at SYRMEP (Synchrotron Radiation for Medical Physics) beamline at the ELETTRA Synchrotron Facility in Trieste, Italy. The beamline provides a monochromatic laminar-section X-ray with a maximum area of 160×5 mm^2 at 20 keV, at a distance of 23 m from the source. The monochromator system consists of a Si (111) crystal working on Bragg configuration. The useful energy range is 8–35 keV and the intrinsic energy resolution of the monochromator is about 10^{-3}. Typical flux measured at the sample position at 17 keV is about 1.6×10^8 photons/mm^2/s with a stored electron beam of 300 mA as ELETTRA operates at 2 GeV (Abrami et al., 2005).

The detector system included a 12/16 bit CCD camera composed of a very high resolution system (4008 horizontal $\times$ 2672 vertical pixels) with a full frame CCD imager, which is directly bonded to a tapered fiber optic with an active input area of 18 (h) $\times$ 12 (v) mm^2 on the sensor. A GadOx scintillator has been deposited directly onto the front surface of this tapered optic. The scintillator is optimized for resolution with X-ray energies in the range 5–35 keV. The pixel size is 4.5 μm with input taper ratio of 1:2 (Magnifying). The detection system was positioned at 6 cm away from the sample position so that absorption technique could be performed. The projections were acquired on a range from 0° to 180°, in steps of 0.15°, resulting in 1200 projections. A micrometric vertical and horizontal translation stage allows the positioning and the scanning of the sample with respect to the stationary beam and a rotational stage, with a resolution of 0.001° allows the acquisition of the projections. A custom-built ionization chamber, placed upstream to the sample, is used to determine the exposure on the sample, and hence to calculate the delivered dose. The energy was set to 21 keV and the exposure time was about 1.9 s per projections and a system resolution of 9.0 μm.

The 2D projections were normalized by using flat (images without the sample placed in front of the beam) and dark (images with the beam shut off) images. This procedure allows one to take into account incident beam non-uniformities and to correct fixed noise due to the efficiency of the detector elements. Tomographic raw images were reconstructed using an imaging processing software (SYRMEP TOMO PROJECT) developed in the SYRMEP laboratory (Montanari, 2003) which uses Interactive Data Language (IDL). The reconstruction was performed using filtered back-projection with Shepp Logan filter. The quantitative analysis was performed in four regions of interest (VOI) within each femur diaphysis ($20 \times 20 \times 350$ pixels) inside the intracortical region of the same sample, starting 0.1 mm below the bottom of the proximal metaphysic of the femur. BoneJ software (ImageJ plugin) (Doube et al., 2010) was employed for quantifying the samples. All 3D images were made using Avizo Standard® 8.0 software.

2.3. Morphometrical parameters

The 3D reconstructed data are a collection of attenuation coefficients distributed regularly into the space. Once a 3D map of the bone specimens is achieved utilizing microtomography, the specimen is fragmented into voxels, each one representing a single solid. Prior to quantification of morphometrical parameters, the images have to be segmented so that voxels corresponding to the bone could be distinguished from those of the background. To distinguish bone tissue from the background, an optimal threshold for each specimen was determined. The method used is implemented in ImageJ, called IsoData, also known as Iterative Intermeans (Riddler and Calvard, 1978). In this work, four regions containing the same volume (VOI) were evaluated, and then an average of these values was calculated for each sample.

In a binary image, BV is the number of white voxels while TV is the total number of voxels in the analyzed volume inside femur diaphysis. Cortical porosity (Por.Ct) reflects the relative volume of porous (Por.V) within the sample tissue volume (TV). This value is the complement of bone volume fraction (BV/TV) and is analogous to marrow volume fraction in trabecular bone (Ma.V/TV). Likewise,