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Effects of chronic lead exposure on bone mineral properties in femurs of growing rats

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ABSTRACT

Lead exposure has been associated with several defective skeletal growth processes and bone mineral alterations. The aim of the present study is to make a more detailed description of the toxic effects of lead intoxication on bone intrinsic material properties as mineral composition, morphology and microstructural characteristics. For this purpose, Wistar rats were exposed (n = 12) to 1000 ppm lead acetate in drinking water for 90 days while control group (n = 8) were treated with sodium acetate. Femurs were examined using inductively coupled plasma optical emission spectrometry (ICP-OES), Attenuated Total Reflection Fourier transform infrared spectroscopy (ATR-FTIR), X-ray diffraction (XRD), and micro-Computed Tomography (μCT). Results showed that femur from the lead-exposed rats had higher carbonate content in bone mineral and $(Ca^{2+} + Mg^{2+} + Na^{+})/P$ ratio values, although no variations were observed in crystal maturity and crystallite size. From morphological analyses, lead exposure rats showed a decreased in trabecular bone surface and distribution while trabecular thickness and cortical area increased. These overall effects indicate a similar mechanism of bone maturation normally associated to age-related processes. These responses are correlated with the adverse actions induced by lead on the processes regulating bone turnover mechanism. This information may explain the osteoporosis diseases associated to lead intoxication as well as the risk of fracture observed in populations exposed to this toxicant.

1. Introduction

Bone is a biological composite formed by a mineral phase, mainly carbonated apatite, and an organic matrix, mostly collagen. Bone mineral is hierarchically ordered on a specific spatial configuration and structure from nanometer to centimeter scale (Olszta et al., 2007). Bone formation and growth is controlled by a complex array of feedback processes that depends on several biological and environmental factors (i.e. exposure to toxicants). A number of toxicological studies have demonstrated that bone tissue is highly sensitive to many types of toxic substances (i.e., heavy metals, organochlorine compounds) which affect bone composition and mineralization, producing specific bone abnormalities and pathologies (Álvarez-Lloret et al., 2009; Hodgson et al., 2008; Rodríguez-Estival et al., 2013; Rodríguez-Navarro et al., 2006). Environmental Pb exposure has been associated with retarded skeletal growth (Berlin et al., 1995). As well, several studies in laboratory model have also described different pathological processes in bone tissue due to lead toxicity, as evidenced by decreased trabecular bone volume and thinner growth plate cartilage in growing laboratory animals (Conti et al., 2012b). In addition, Pb intoxication has been also shown to impair bone biomechanics and structure in ovariectomized rats, suggesting a deleterious effect of the metal in older stages of life (Lee et al., 2016).

Lead poisoning may exert both direct and indirect actions on the processes regulating bone turnover mechanisms (Berglund et al., 2000). These alterations are related to the compatibility of Pb^{2+} to substitute Ca^{2+} in the apatite lattice and, consequently, altering the homeostasis of calcium in bone metabolism. Moreover, lead exposure may alter hormonal regulation of organisms and directly damage bone-cells functions (Berglund et al., 2000). Previous studies have shown how lead exposure can alter the bone

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mineral composition and affect bone maturation and skeletal growth (Monir et al., 2010; Rodríguez-Estival et al., 2013). Furthermore, many researches also demonstrated that increased lead exposure is also associated with decreased bone mineral density as well as detrimental bone strength (Escribano et al., 1997; Ronis et al., 2001). Lead intoxication is known to induce higher risk of fractures as it affects several biomechanical properties, such as maximal load supported at fracture and energy absorption capacity by the whole bone. Additionally, geometrical properties are also impaired after lead exposure, as reported by a diminished diaphyseal bone marrow medullar cavity in femur of growing rats (Conti et al., 2012a, 2012b). As there is an intimate connection between bone composition and morphology and bone biomechanical properties, the mineral composition should be taken into consideration when analyzing Pb effects on bone structure. It is also well known that lead, once absorbed into the body, is mainly stored (more than 95%) in the mineralized tissues (i.e., teeth and bones). Moreover, lead accumulation will occur predominately in trabecular bone during childhood, and in both cortical and trabecular bone in adulthood (Auf der heide and Wittmers, 1992). Therefore, the effects of lead accumulation in bone can be expressed in different ways in these regions determining the diverse response to chronic poisoning.

Although there is a high interest in the study of the metabolic and physiological alterations caused by lead exposure in organisms, few studies have focused on the detailed analysis of the changes produced by this exposure in bone tissue at mineral level and its properties. Several effects have been described on bone mineralization due to environmental lead exposure in species of ungulates and birds through different acquisitions and sources (Álvarez-Lloret et al., 2014; Gangoso et al., 2009; Rodríguez-Estival et al., 2013). Controlled animal studies have also showed significant alterations on bone mineralization in lead exposure in vivo models (Lee et al., 2016; Monir et al., 2010). In this sense, several analytical techniques are available to study the inorganic properties of bone tissue that also allow to identified specific toxic lead effects due to pathological conditions affecting bone mineralization.

The purpose of this research was to conduct a comprehensive analysis of the bone mineral composition, morphological and microstructure characteristics in femurs to study the effects of chronic lead poisoning in a Wistar rat model. The present study was designed with the same experimental model as the one proposed in previously reported studies from our laboratory (Conti et al., 2012a, 2012b) in which morphological alterations were observed but the material properties were not deeply investigated. For this purpose, we have employed a combination of analytical techniques including: inductively coupled plasma optical emission spectrometry (ICP-OES), Attenuated Total Reflection Fourier transform infrared spectroscopy (ATR-FTIR), X-ray diffraction (XRD), and micro-Computed Tomography (μCT) analyses to fully characterize bone mineral properties. The combined use of these techniques can provide detailed compositional and structural information of bone at different scales. This information may help us to define the specific mechanisms behind the toxic effects on bone mineralization provoked by lead exposure.

2. Materials and methods

2.1. Animal treatment and sample preparation

Twenty female Wistar rats aged 21 days from the animal facility of the Faculty of Pharmacy and Biochemistry, Buenos Aires University (Argentina), were used throughout the experiments. They were housed in stainless-steel cages and maintained under local vivarium conditions (temperature 22–23 °C, 12-h on/off light cycle). All animals were allowed free access to tap water and a standard Pb free pelleted chow diet. Tap water quality is in accordance with international guidelines and standards ISO and WHO references being the Pb content less than 0.005 ppm. Rats were randomly divided into 2 groups. Pb intoxication was induced in 12 animals through administration of 1000 ppm of lead acetate in the same tap water for 90 days (Hamilton and O'Flaherty, 1994). Control animals (n = 8) received equivalent acetate, as sodium acetate, added to the mentioned water. All animals were treated in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals (NIH 8th edition, 2011). Protocols were approved by the Ethical Commission of the Faculty of Dentistry, University of Buenos Aires (N° 11/06/2012–23). Body weight and femur length were registered at the beginning of the experiment (day 0) and at the end of the experimental period (day 90). Animals were euthanized by unconscious decapitation and femurs were properly dissected, cleaned from soft tissue, and stored at −20 °C until analyses. For ICP-OES, ATR-FTIR, and DRX analyses bone samples were previously powdered using a cryogenic mill (CertiPrep 6750 Freezer/Mill, SPEX).

2.2. Elemental analyses

For the elemental analyses, 30 mg of bone powder was dissolved in a 10 ml solution of 70% HNO_3 (1 ml, 24 h) and 30% H_2O_2 (1 ml, 24 h) and microwave digested. Calcium, phosphorus, magnesium, sodium and lead concentrations were measured using an Optima 8300 ICP-OES (Perkin Elmer). Concentrations are given in dry weight (d.w.). The precision of chemical analyses was higher than 1 ppm.

2.3. Fourier transform infrared (ATR-FTIR) spectroscopy

For the ATR-FTIR analyses, infrared spectra were obtained on a FT/IR-6200 spectrometer (JASCO, Japan). Infrared spectra were collected from 600 to 4000 cm^{-1} in absorbance mode, 124 scans at 1 cm^{-1} resolution. All curve fitting was performed and integrated areas measured using the curve fitting software JASCO Spectra Manager ® and PeakFit v4.11 Systat Software Inc. Two different IR bone absorption regions (900–1200 cm^{-1} and 1300–1750 cm^{-1} bands) were analyzed by curve fitting for control and lead-exposed groups (Fig. 1). The amount of phosphate, carbonate and collagen in bone samples were estimated from the peak area of each absorption bands associated with phosphate, carbonate, amide and C—H aliphatic groups identified in the infrared spectra (Boskey et al., 1998; Boskey and Mendelsohn, 2005; Gadaleta et al., 1996; Paschalis et al., 1996; Rey et al., 1989). Overlapping peaks were resolved using a second derivative methodology and fitted to a mixed derivative Gaussian + Lorentzian function. Fig. 2 displays the curve-fitting analysis for average IR spectra for control group in the 900–1200 cm^{-1} (phosphate absorption bands) and 1300–1750 cm^{-1} (carbonate type B substitution and amides absorption bands). To minimize the effect of differences in sample size, peak areas were normalized to the area of 3800–2800 cm^{-1} band region associated with OH groups after removing the C—H stretching peak area from this region. All data were baseline corrected and expressed as intensities ratios. A detailed description of the methodology used in described elsewhere (Rodríguez-Navarro et al., 2006). The following parameters derived from the ATR-FTIR spectra analyses were calculated to describe bone mineral composition and crystallinity index parameters. The degree of bone mineralization was defined as the band intensity ratios of phosphate species in the bone mineral to organic matrix ratio (Pienkowski et al., 1997) and was estimated as follows: A900-1200/A1660; where A900-1200 represent the amount of phosphate in bone and A1660 the amount of amide I groups (main band from